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GENETIC CHARACTERIZATION OF MAIZE INBRED LINES AND THEIR MUTUAL AFFINITIES

ABSTRACT

Twenty-five inbred lines of maize assigned to six heterotic groups and developed in Poland were studied electrophoretically to determine their purity and mutual relationships. The electrophoretic data were analysed by principal component and cluster analyses. All lines (except five) were monomorphic in each enzyme studied. Electrophoretic affinities of inbred lines were, with some exception, were in accordance with the expectation inferred from their pedigrees. Lines of identical pedigree but with small differences in phenotypic or developmental characteristics had the lowest dissimilarity coefficient values and in cluster and principal component analyses behaved accordingly. They showed however diverse levels of clustering and dissimilarity coefficient values, which most likely reflected their departure from the common parental gene pool, which occurred in the course of breeding manipulations. Lines, which had one parent in common, were less similar to each other than those with identical pedigree. The most distinct electrophoretically were lines with complex or dissimilar pedigree formulae as was confirmed by cluster and principal component analyses.

Key words: Inbred lines, enzymes, electrophoresis, similarity, pedigree, heterosis, maize

INTRODUCTION

Genetic characterization provides means of assessing the wealth of germplasm diversity both in cultivation and in breeding stock and is instrumental for identification and verification of line pedigrees and affinities between lines and varieties. This important information is often used for selecting components to be used in breeding scheme and is becoming important for registration, plant variety protection (PVP), and utility patents (Smith 1988, Smith 1989, Orman *et al.* 1991). High heterosis effect is a target for maize breeders. Lines with remote affinity are expected to generate high heterotic effects in their hybrids. In order to facilitate breeding processes scientists are challenged to develop accurate methods to identify combinations of maize lines with potential to produce highly heterotic hybrids. Isozymes as well as DNA markers have been extensively

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investigated for their utility (Frei *et al.* 1986, Ajmone-Marsan *et al*. 1992, Ajmone-Marsan *et al.* 1998).

The aim of this study was to determine purity and relationships of inbred lines, organized by breeders in six heterotic groups, vis a vis their pedigrees. This was our preliminary study leading to a broader survey of application of enzyme markers in prospecting heterotic effect to be generated by inbred lines in hybrids.

MATERIAL AND METHODS

Seed material of twenty-five maize inbred lines classified into six heterotic groups: Iowa Dent (I), Lancaster Sure Crop (L), Stiff Stalk Synthetic (S), 354, Co 255 (CO), Others (R), on the basis of their pedigrees and physiological and phenotypic characteristics, was provided for this study by the Plant Breed-

Table 1

Characteristics of inbred lines used

Line	Endosperm type	Pedigree	Group allocation (abbreviation)
I245	Dent	P3906 hybrid	Iowa Dent (I)
I336	Dent	Iowa Dent × P3950 selected	Iowa Dent (I)
I336A	Dent	Iowa Dent × P3950 selected	Iowa Dent (I)
I41789	Dent	Iowa Dent × S336	Iowa Dent (I)
I41806A	Dent	Iowa Dent × S336	Iowa Dent (I)
L335	Dent	Mo17 × CM7/22970-5	L/CM7 (L)
L350	Dent	S149 × Mo17	L/354 (L)
L39171	Dent	(FR22 × Mo17) × S162	L (L)
S208z	Dent	P3732 hybrid	SSS/L or I (S)
S208r	Dent	P3732 hybrid	SSS/L or I (S)
S339	Flint	(B73 × F2) × F2	SSS/F2 (S)
G126	Dent	Canadian Gene Pool-1	354 (G)
G160	Dent	Canadian Gene Pool-2	354 (G)
G359	Dent	S160 × S126	354 (G)
G359A	Dent	S160 × S126	354 (G)
G360	Dent	S149 × Mo17	354/L (G)
Co255	Flint	F7 × EP1/W33 × F115	Co 255 (Co)
Co311	Flint	(MK3 × Co255) × Co255	Co 255/L (Co)
Co35438	Flint	(F2 × LV4) × Co255	Co 255/F2 (Co)
Co35439	Flint	(F2 × LV4) × Co255	Co 255/F2 (Co)
R152	Dent	Canadian Gene Pool-21	Other, L? (R)
R2570S	Flint	German flint	Other (R)
R41317A-3	Flint	German flint × GK72-74	Other (R)
R41324A-2	Flint	German flint × GK72-74	Other (R)
R41336	Flint	German flint × GK72-74	Other (R)

ing Smolice Ltd in Poland. Selection of lines, allocation into groups and the summary of their pedigree is shown in Table 1.

Lines were assayed electrophoretically for 10 enzyme systems namely acid phosphatase-ACP (E. C.3.1.3.2), alcohol dehydrogenase -ADH (E. C 1.1.1.1), aspartate aminotransferase-AAT (E. C.2.6.1.1), catalase-CAT (E. C.1.11.1.6), diaphorase–DIA (E. C.1.6.99.), esterase -EST (E. C. 3.1.1, isocitrate dehydrogenase–IDH (E. C.1.1.1.42), malate dehydrogenase-MDH (E. C.1.1.1.42), phosphoglucomutase -PGM (E. C.5.4.2.2) and phosphogluconate dehydrogenase-PGD (E. C.1.1.1.44).

Five day old coleoptiles of 36 plants per inbred line were assayed. For the enzyme extraction 8.3% sodium ascorbate solution of pH 7.4 was used. Electrophoretic runs were developed in three buffer systems: 0.036 M. Lithium-borate pH 8.3, 0.072 M histidine-citric acid pH 6.5 and 0.074 M histidine-citric acid pH 5.0 buffers. Buffer systems and procedures of electrophoresis followed those of Stuber *et al.* (1988).

Each line was scored for presence or absence of all alleles occurring in each enzyme system to produce binary data matrix, which was subsequently used to analyse genetic similarities between lines by principal component (PCA) and cluster analyses using Statistica for Windows (1995). Clustering was performed with the Ward algorithm using Euclidean distance squared matrix.

RESULTS

All lines, except five, were monomorphic but often fixed for alternative alleles in enzyme loci. Lines G359, I41789, I41806A, I245 and R152 showed some insignificant heterozygosity in PGD, IDH, MDH and PGM and ACP enzymes accordingly, which was not taken into account in statistical analyses.

In cluster analysis 14 lines belonging to I, G, and R groups clustered according to their allocation into groups unlike remaining 11 lines assigned to Co, L and S groups (Fig 1). Euclidean Distance Squared values for pairs of lines (Table 2) revealed details of associations between lines. Some lines of L and S groups had more affinity to members of other groups then to lines of their own group. Similar results to those of cluster analysis were obtained from the PCA (Fig. 2). Table 3 provides values of correlation (factor loadings) of inbred lines with first two components of PCA. Lines G.359, G 359A, CO35439 and all lines of R group had correlation values above 0.700 with first component. The second component was significantly correlated (values above 0.700) with lines I245, I 336, I 336 A and L 39171. On the first component which accounted for 49.2% of the total variance to which ACP, MDH, IDH, contributed the most lines belonging to R and I groups were separated from the others but the former had close associations with two lines from Co- and three from G groups. Lines of the I group were separated on the second component, which accounted for

Table 2

Euclidean distance squared values for pairs of inbred lines

Line	I245	I336	I336 A	I41789	I41806A	L350	L335	L39171	S208z	S208r	S339	G160	G126
I245	0.0												
I336	10.0	0.0											
I336A	9.0	3.0	0.0										
I41789	14.0	10.0	13.0	0.0									
I41806A	10.0	6.0	9.0	6.0	0.0								
L350	9.0	11.0	10.0	11.0	7.0	0.0							
L335	12.0	12.0	13.0	16.0	12.0	7.0	0.0						
L39171	13.0	9.0	12.0	9.0	11.0	16.0	9.0	0.0					
S208z	14.0	6.0	7.0	12.0	8.0	5.0	8.0	13.0	0.0				
S208r	14.0	6.0	7.0	12.0	8.0	7.0	8.0	11.0	2.0	0.0			
S339	11.0	17.0	16.0	19.0	15.0	16.0	15.0	14.0	17.0	15.0	0.0		
G160	17.0	11.0	14.0	9.0	9.0	8.0	13.0	14.0	7.0	9.0	20.0	0.0	
G126	14.0	12.0	9.0	14.0	10.0	5.0	12.0	19.0	6.0	8.0	17.0	5.0	0.0
G359	18.0	14.0	15.0	16.0	12.0	9.0	12.0	17.0	8.0	8.0	15.0	7.0	6.0
G359A	18.0	14.0	15.0	16.0	12.0	9.0	12.0	17.0	8.0	8.0	15.0	7.0	6.0
G360	13.0	15.0	14.0	15.0	11.0	4.0	7.0	16.0	9.0	9.0	16.0	8.0	5.0
CO255	13.0	11.0	14.0	13.0	13.0	16.0	13.0	8.0	15.0	13.0	10.0	14.0	17.0
CO311	14.0	10.0	11.0	16.0	12.0	9.0	4.0	9.0	4.0	4.0	13.0	11.0	10.0
CO35438	15.0	15.0	16.0	17.0	13.0	10.0	9.0	14.0	9.0	9.0	14.0	12.0	11.0
CO35439	19.0	15.0	16.0	13.0	13.0	10.0	13.0	18.0	9.0	9.0	18.0	12.0	11.0
R152	14.0	10.0	11.0	12.0	8.0	5.0	6.0	11.0	4.0	4.0	15.0	9.0	8.0
R41336	17.0	11.0	14.0	13.0	13.0	12.0	9.0	14.0	7.0	7.0	16.0	12.0	13.0
R41317A	14.0	14.0	15.0	12.0	12.0	9.0	12.0	17.0	8.0	8.0	13.0	11.0	10.0
R41324A	17.0	13.0	14.0	13.0	15.0	12.0	11.0	14.0	7.0	7.0	16.0	12.0	13.0
R2570S	16.0	16.0	17.0	14.0	14.0	11.0	16.0	21.0	10.0	10.0	17.0	13.0	12.0

Table 2

Continued

Line	G359	G359A	G360	Co255	Co311	Co35438	Co35439	R152	R41336	R41317A	R41324A	R2570S
I245												
I336												
I336A												
I41789												
I41806A												
L350												
L335												
L39171												
S208z												
S208r												
S339												
G160												
G126												
G359	0.0											
G359A	0.0	0.0										
G360	5.0	5.0	0.0									
CO255	13.0	13.0	16.0	0.0								
CO311	8.0	8.0	9.0	11.0	0.0							
CO35438	9.0	9.0	10.0	10.0	5.0	0.0						
CO35439	9.0	9.0	10.0	14.0	9.0	4.0	0.0					
R152	6.0	6.0	5.0	13.0	4.0	7.0	7.0	0.0				
R41336	12.0	11.0	12.0	16.0	7.0	12.0	8.0	7.0	0.0			
R41317A	9.0	8.0	9.0	15.0	8.0	9.0	5.0	6.0	3.0	0.0		
R41324A	11.0	11.0	12.0	16.0	7.0	12.0	8.0	7.0	2.0	3.0	0.0	
R2570S	12.0	12.0	13.0	19.0	12.0	9.0	5.0	10.0	7.0	4.0	7.0	0.0

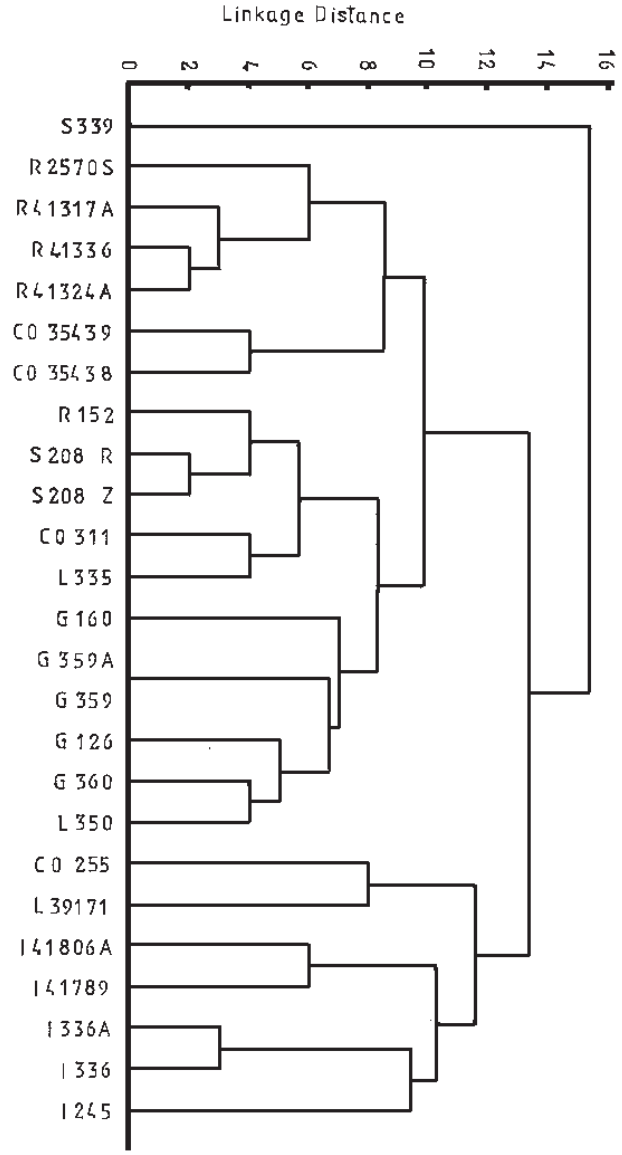

Fig. 1 Associations among inbred lines revealed by cluster analysis of enzyme data

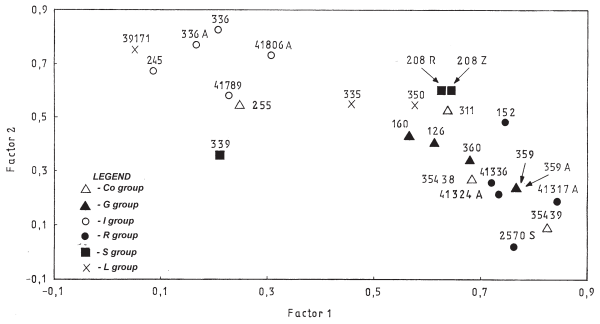

Fig. 2 Associations among inbred lines revealed by PCA of enzyme data

Table 3.

Values of correlation (component loadings) between inbred lines of maize and two first components of PCA.

Line	Component loadings (marked loadings are> 0.700)	
	Component 1	Component 2
I 245	0.085	0.675
I 41789	0.227	0.584
I 41806A	0.307	0.733 *
L 350	0.577	0.546
L 335	0.457	0.550
L 39171	0.051	0.753 *
S 208z	0.639	0.591
S 208r	0.626	0.596
S 339	0.210	0.357
G 160	0.550	0.429
G 126	0.614	0.405
G 359	0.767 *	0.234
G 360	0.680	0.340
Co 255	0.249	0.552
Co 35438	0.685	0.269
Co 35439	0.826 *	0.089
R 152	0.746 *	0.484
R 41324A	0.735 *	0.215
R 2570S	0.763 *	0.021
I 336	0.207	0.827 *
G 359A	0.767 *	0.234
Co 311	0.639	0.526
R 41336	0.720 *	0.258
R 41317A	0.844 *	0.189
I 336A	0.166	0.772 *

9.6% of the total variance To this component IDH, ADH, CAT and DIA contributed the most. The distinction of G lines was not as evident in PCA as it was in cluster analysis.

DISCUSSION

Electrophoretic similarities of inbred lines were, with some exceptions, in accordance with expectations inferred from their pedigrees. Lines developed from the same parental stock but showing small differences in phenotypic or developmental characteristics such as I 336 and I 336A, I 41789 and I41806A, S 208R and S 208Z, G 160 and G 126 and G 359 and G 359A, Co 35438 and Co 35439, R 41336, R-41324A-2 and R 41317A-, or L350 and G 360 had the lowest dissimilarity coefficient values. They also performed accordingly in cluster and principal component analyses. Different values of dissimilarity coefficient and diverse levels of clustering for pairs of lines with identical pedigree reflect their departure from the common parental gene pool which occurred in the course of breeding manipulations. Lines which had only one common parent such as I 336, I 336A and I 41789, I 41806A or R 41336, R 41324A-2 and R 41317A-3, showed relatively lower level of similarity whereas lines with complex or distinct pedigree were the most distinct electrophoretically. The latter case was observed for lines of S, Co and L groups and is well exemplified by Co311, Co35438 and Co35439 lines, which contain various quantity of Co 255 germplasm as an effect of elaborated genetic manipulations. The close affinity of these three lines with Co 255 is further evidenced by low hetereosis effect in hybrids produced between aforementioned lines and Co 255. It leads to conclusion that electrophoretic evidence seems to have limited application for investigating affinities between lines whose origin is complex and includes a range of different germplasm.

In the R group consisting of 4 lines, only one R152 was unrelated to other members of the R group on pedigree grounds what was also confirmed by electrophoretic evidence from this study. This line unlike the other R lines had kernels of dent type and showed the most electrophoretic similarities with S208R and S208Z lines from S group. Both these latter lines had no genetic affinity with R152 as judged by their pedigrees, but they also produced kernels of the dent type.

Similar close electrophoretic association was observed between S 339 and Co 255 lines (Table 2 and Fig. 2) both producing kernels of flint type. They belong however to two different heterotic groups, with remote genetic affinity. These two latter cases suggest that the difference between type of kernels has strong and complex genetic basis. Such different lines are usually good components to produce highly heterotic hybrids, as each kernel type is associated with different range of genetic variation. In Central Europe such hybrids are commonly produced as they better tolerate low temperature in the spring and

their grains reach physiological maturity in relatively short time (Sowiński *et al.* 1998). It has also become a common practice to integrate germplasm of these two types of grain in one inbred line. Co 255 and L335 and S339 are examples of such practice and because of that, grain type itself can not be used as a criterion for identifying hybrid components.

Similar reservation is pertinent to geographical origin of germplasm, which often is assumed to be a good indicator of plant characteristics (Adamczyk 1999). Line S339, which represents a very remote gene pool relative to those of other inbred lines considered here (Figs 1 and 2) is a backcross to the parent with kernels of flint type. Thereby it retained kernels of flint type despite its other characteristics being in common with those of other members of S group. This line contributed new traits to the range of germplasm being utilized in Polish maize breeding programmes and it has been successfully included into recently developed high-yielding hybrid variety Polan.

In conclusion, results of this study provided useful guidance for further investigation of presented methodology with a view of its use for prospecting line performance in the developing maize highly heterotic hybrids. The application of electrophoretic evidence however, for elucidating affinities of inbred lines decreases with complexity of breeding manipulations performed in the course of their development and inclusion of a variety of germplasm into genetic makeup of such lines.

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