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GENETIC VARIABILITY AND DIVERSITY OF CORN BREEDING MATERIAL ORIGINATING FROM DOMESTIC AND FOREIGN POPULATIONS DETERMINED ON THE BASIS OF BIOCHEMICAL-GENETICAL MARKERS

ABSTRACT: Isozymes can serve as genetic markers and their number should be large enough in order to make the coverage of genomes as complete as possible and in order to use these methods for gene marking for required agronomic traits. These markers are the products of 21 mapped genes, which is relatively reliable number for their application in mapping for certain agronomic traits. Genetic variability and diversity are significant for populations and for selfpolinated lines as basic material in breeding and creation of new corn hybrids. For that reason, several groups of corn populations of different origin were analyzed. Two groups of Yugoslav populations, Italian, Portuguese and French collections were assessed on the basis of detected alleles of 21 loci and standard genetic distances between genotypes. Yugoslav corn collections had shown high heterozygosity, on the basis of isozymes as gene markers. Genetic diversity of Italian populations was pronounced on the basis of some loci, and the Portuguese populations had more polymorphic and more heterozygous loci than French populations. Inter-genetic variability between populations and their geographical location are very important in breeding crops for creation of heterosis.

KEY WORDS: Genetic variability, diversity, genetical markers, isozymes, corn, populations.

INTRODUCTION

Biochemical, physiological and genetical studies should be connected at the level of gene, i.e. molecular level. Research and application of new molecular methods should, above all, find its way in breeding and seed science. Application of molecular markers is multilateral and is used for:

— identification of genes for desirable agronomic traits
— identification of genes for disease resistance
— identification of genes for qualitative seed traits

Role of molecular markers in breeding is based on „linkage" of gene markers and genes for required quantitative and qualitative traits. This kind of research is widely used in the world. They encompassed laboratory analysis of markers and integration of these methods with classical breeding methods. These methods can be applied in seed science, in control of specific seed quality, i.e. genetic purity. For their application the wide spectra of methods by which screening tests are made possible should be introduced.

Spectra of genetic changes of cultivated species and their wild relatives are kept and maintained in seed banks and field gen banks world widely (Simpson, Withers, 1986). Markers in gene-banks have potential application in identification of collection samples and are different among tested samples, clones, pure lines, inbreeding of population groups which differ in genetic variability and demand different treatments.

The aim of this investigation was to determine genetic variability and diversity of breeding corn material of different origin on the basis of isozymes as gene markers. Used isozymes were products of certain polymorphous loci on the basis of which some genetic characteristics of great number of populations of certain groups were determined as well as their significance for creation of heterosis hybrids.

According to many authors (Salanoubat, Pernes, 1986, Veldboom, Lee, 1996), result of adaptation process to biotic and abiotic is highly heterogeneous population. Modifications of pure lines are according to Hallauer (1990) continued with new sources of germplasm. Methods for parent identification, and for heterozygous pairs crossing, are the molecular markers, according to the same author. Isolation and identification of DNA sequences and genes, are meant to be efficient tools for development of lines and identification of best crossing. Genetic classification of lines originating from different population groups can be done on the basis of molecular markers far more efficient than on the basis of field testing of genotypes of unknown heterosis effect (Mumm, Dudley 1994). This type of estimation is made on the basis of isoenzymic markers for hybrid identification, for efficient selection, and discovering the genetic traits of elite hybrids (Smith, 1989.)

Usage of molecular markers is being introduced into a basis of genetic researches by which all components of breeding are connected and have a key role in genetical, biochemical, physiological and molecular basis of heterosis (Smith and Chin, 1993).

The aim of this investigation was to determine genetic variability and diversity of breeding corn material of different origin on the basis of isozymes were products of certain polymorphous loci on the basis of which some genetic characteristics of great number of populations of certain groups were determined as well as their significance for creation of heterosis hybrids.

MATERIAL AND METHODS

Several groups of corn populations of different origin were analyzed. Yugoslav populations encompassed two groups: 17-hard dents and 18-soft flints.

These populations were obtained by hybridization of populations belonging to other groups.

Collections from Italy came from different regions of this country, 50 populations from the collection of Portuguese and 20 populations from French collection. These populations originated from different geographical and ecological regions and they differed in vegetation period.

Genetic characters of the populations were assessed on the basis of allozymic genotypes belonging to 20 loci. The tested materials were analyzed for the frequency of detected alleles, polymorphism and heterozygosity of the loci and standard genetic distances after N e i (1978). Genetic diversity between populations of certain groups was determined by cluster analysis according to Euclidean distances.

Application of genetic markers, their hromozomic location and the methods of reading were done according to S t u b e r et al. (1988) on the basis of polymorphism of enzymic systems and 20 loci.

RESULTS AND DISCUSSION

Domestic populations from two groups: 17-hard dents and 18-soft flints were analyzed on the basis of 21 isoenzymic loci on which 66 alleles were found (Tab. 1). Populations of these groups were obtained by hybridization of populations belonging to some other groups and that is way they are considered to be the „youngest". On the basis of certain loci (Mdh1,2, Adh1, Got2) differences, between populations and groups to which they belong, were found.

Tab. 1 — Alleles detected in examined Yugoslav collections

No.	Loci	Allele-designated	Total
1.	Acp 1	2, 3, 4, 6	4
2.	Adh 1	4, 6, N	3
3.	Cat 3	7, 9, 12, N	4
4.	Enp 1	6, 7, 8	3
5.	Est 8	3, 4, 4.5, 5, 6	5
6.	Glu 1	1, 2, 3, 6, 7, N	6
7.	Got 1	4, 6	2
8.	Got 2	2, 4, 6, N	4
9.	Got 3	4	1
10.	Idh 1	4, 6, N	3
11.	Idh 2	4, 6	2
12.	Mdh 1	1, 6, 9, 10.5, N	5
13.	Mdh 2	3, 3.5, 6, N	4
14.	Mdh 3	16, 18	2
15.	Mdh 4	8, 12	2
16.	Mdh 5	12, 15	2

17.	Pgm 1	9, 16, 17, N	4
18.	Pgm 2	2, 3, 4	3
19.	Pgd 1	2, 3.8	2
20.	Pgd 2	2.8, 5	2
21.	Phi 1	3, 4, 5	3
			Total: 66

Genetic diversity of the populations inside groups was analyzed on the basis of standard genetic distance (D). For these groups the cluster analysis was given (Fig. 1 and 2). The populations C133; C432, C214 and C433 were similar in group 17. In this group division of populations into two groups, the first one from C133 to C76 which was more homogenous, and the second from C612 to C382, was noticed. In group 18 the similar populations were C139, C576, C533, C647 and C326. In this group two populations were distinguished from the rest of the analyzed populations in this group (C124 and C127) which were genetically rather distant. In order to broaden the genetic basis for corn breeding the populations with greater variability and diversity, although these two factors can, but don't necessarily have to be in correlation, can be of some significance.

The populations from Italy gathered at the Istituto Sperimentale per la Cerealicoltura, Bergamo, were analyzed. Thirty (30) populations from different regions of Italy were studied. Genetic properties of the populations were determined on the basis of analyzed eleven enzymic systems controlled by twenty (20) loci. The alleles in these populations were detected, their frequencies and heterozygosity of the studied loci was determined, and finally the standard genetic distance among all analyzed populations i.e. genetic diversity between all pairs of populations were determined (Tab. 2).

Tab. 2 — Alleles, polymorphism of Loci, Heterozygousity and Standard Genetic Distances (D) in Italian Populations

Number of population	Number of alleles	Average alleles per locus	Mean proportion of polymor. loci (%)	Heterozyg ous. (He)	Mean of D
1	33	1.6	55	0.173	0.072
2	32	1.6	45	0.168	0.076
3	32	1.6	45	0.129	0.067
4	34	1.7	60	0.224	0.080
5	31	1.5	50	0.175	0.076
6	32	1.6	50	0.169	0.091
7	33	1.6	45	0.181	0.058
8	34	1.7	55	0.185	0.067
9	37	1.8	60	0.212	0.058
10	34	1.7	60	0.203	0.063
11	33	1.6	55	0.171	0.068
12	31	1.5	50	0.202	0.073
13	33	1.6	55	0.223	0.046

14	32	1.6	50	0.209	0.070
15	30	1.5	45	0.179	0.069
16	38	1.9	70	0.242	0.072
17	41	2.0	75	0.235	0.069
18	35	1.7	55	0.220	0.055
19	34	1.7	45	0.212	0.059
20	37	1.8	60	0.212	0.075
21	36	1.8	60	0.220	0.048
22	35	1.7	55	0.207	0.054
23	36	1.8	60	0.232	0.057
24	31	1.5	50	0.218	0.056
25	35	1.7	50	0.217	0.044
26	33	1.6	50	0.292	0.058
27	35	1.7	55	0.182	0.054
28	32	1.6	50	0.190	0.053
29	31	1.5	50	0.222	0.070
30	32	1.6	45	0.200	0.063

The comparison of the frequencies of alleles in Italian and Yugoslav maize populations showed that frequencies for most alleles were similar, but there were alleles which frequencies were different in these collections. On the basis of some alleles which were rare or more frequent in the populations some differences were found among Italian and Yugoslav maize collections (Acp1, Adh1, Mdh5).

The mean polymorphism of the analyzed loci was 45 to 75% (Tab. 2). On the basis of the obtained frequencies of the detected alleles, the genetic distances (D) were calculated for all analyzed populations. Standard genetic distance ranged from 0.044 to 0.091.

Open pollinated corn collections were examined, 50 from Portuguese and 20 from French collections. The populations originated from different geographical and ecological regions. Their genetic characters were assessed on the basis of isozymes as gen markers controlled by 20 loci. A range of allelic frequencies was detected (Tab. 3). The detected alleles were common to almost all populations and their frequencies were usually high. Several new but rare alleles were found in both collections. These populations differed in frequency of the alleles on loci Acp1, Glu1, Mdh2, Pgd2. The total number of alleles per population was 31 to 43 for Portuguese collection, i.e. an average of two alleles per locus (Tab. 4).

Tab. 3 — Average frequencies for the detected alleles in Portuguese and French collections

Locus-allele	Frequency	Locus-allele	Frequency
Portugal		France	
Acp 1—2	0.45	Acp 1—2	0.44
3	0.03	3	0.01
4	0.49	4	0.56
6	0.03	6	0.01

Adh 1—4	0.65	Adh 1—4	0.65
6	0.35	6	0.35
N	0.01	N	0.01
Cat 3—7	0.06	Cat 3—7	0.11
9	0.62	9	0.64
12	0.28	12	0.24
N	0.04	N	0.01
Est 8—4	0.50	Est 8—4	0.49
4.5	0.49	4.5	0.49
5	0.01	5	0.02
6	0.01	6	0.01
		N	0.01
Glu 1—1	0.01	Glu 1—1	0.01
2	0.20	2	0.18
3	0.06	3	0.08
6	0.05	6	0.02
7	0.61	7	0.44
9	0.01	8	0.01
10	0.01	10	0.01
N	0.08	N	0.26
Got 1—4	0.97	Got 1—4	0.98
6	0.03	6	0.02
		N	0.01
Got 2—2	0.11	Got 2—2	0.13
4	0.89	4	0.87
		N	0.01
Got 3—4	1.00	Got 3—4	1.00
Idh 1—4	0.99	Idh 1—4	0.98
6	0.01	6	0.01
		N	0.01
Idh 2—4	0.64	Idh 2—4	0.44
6	0.36	6	0.56
Mdh 1—1	0.03	Mdh 1—1	0.05
6	0.91	6	0.94
10.5	0.06	10.5	0.01
Mdh 2—3	0.21	Mdh 2—3	0.16
3.5	0.03	3.5	0.02
4.5	0.01	4.5	0.01
6	0.76	6	0.82
Mdh 3—16	0.99	Mdh 3—16	0.99
18	0.01	18	0.01
Mdh 4—12	1.00	Mdh 4—12	1.00
Mdh 5—12	0.80	Mdh 5—12	0.92
15	0.20	15	0.08
Pgm 1—9	1.00	Pgm 1—9	0.99
		16	0.01
Pgm 2—2	0.02	Pgm 2—2	0.09
3	0.07	3	0.05
4	0.91	4	0.86
Pgd 1—2	0.23	Pgd 1—2	0.46
2.8	0.03	3.8	0.54
3.8	0.74		

Pgd 2—2	0.01	Pgd 2—2	0.01
2.8	0.01	2.8	0.01
5	1.00	5	0.98
		10	0.01
		N	0.01
Phi 1—3	0.01	Phi 1—3	0.01
4	0.97	4	0.96
5	0.03	5	0.03

The average heterozygosity of loci in Portuguese populations varied between 0.129 and 0.269, and in French populations between 0.098 and 0.223. The results of the analyses indicate that the Portuguese populations had more polymorphic and more heterozygous loci than French populations (Tab. 4).

Tab. 4 — Polymorphism of loci and heterozygosity in Portuguese and French populations

Population	Mean proportion of loci polymorphism (%)	Hetero-zygosity	Population	Mean proportion of loci polymorphism (%)	Hetero-zygosity
Portugal					
1.	60	0.186	26	60	0.238
2.	50	0.173	27	65	0.246
3.	40	0.129	28	65	0.262
4.	65	0.207	29	65	0.245
5.	65	0.230	30	70	0.239
6.	70	0.225	31	60	0.216
7.	60	0.214	32	70	0.210
8.	70	0.227	33	60	0.195
9.	80	0.241	34	60	0.228
10.	70	0.267	35	55	0.217
11.	60	0.185	36	65	0.229
12.	55	0.231	37	70	0.203
13.	55	0.201	38	60	0.219
14.	60	0.218	39	80	0.234
15.	60	0.209	40	60	0.214
16.	65	0.221	41	55	0.236
17.	60	0.197	42	55	0.225
18.	70	0.222	43	55	0.238
19.	65	0.244	44	60	0.205
20.	65	0.199	45	65	0.228
21.	65	0.214	46	50	0.197
22.	65	0.261	47	65	0.241
23.	70	0.247	48	55	0.195
24.	60	0.223	49	60	0.176
25.	70	0.269	50	75	0.245

France					
1.	45	0.137	11	55	0.201
2.	60	0.200	12	30	0.128
3.	50	0.150	13	55	0.190
4.	60	0.214	14	65	0.180
5.	60	0.211	15	50	0.158
6.	55	0.223	16	50	0.208
7.	60	0.184	17	60	0.134
8.	55	0.195	18	75	0.145
9.	55	0.208	19	35	0.098
10.	45	0.171	20	60	0.208

The significance of inter-genetic variability between populations and link between their structure and geographical location are very important in breeding programs and usage of germplasma collections by breeders (S a l a n o u - b a t , P e r n e s , 1986).

Genetic diversity of corn breeding material and its combining ability can be determined on the basis of several markers such as: morphological, isoenzymic, components of storage proteins, DNA fragments (S m i t h and S m i t h , 1989). Their different possibilities and need for applying different methods were also determined. Advantage of RFLP markers over testing in the field and isozymes come from their greater efficiency and more complete genome coverage. They are better in determining the complete variability identification and diversity of genotypes.

CONCLUSION

— Isozymes as genetic markers are products of genes and as such serve for assessment of starting breeding material i.e. populations which serve as „gene-banks" in the breeding hybrid process.

— Genetic and molecular markers can serve to point out the degree of variability and diversity of breeding material. Creation of hybrids depends on choice of breeding material.

— Yugoslav corn collections had shown on the basis of polymorphism of analyzed loci fairly high heterozygosity. Genetically very similar, but also very distant populations were found.

— Identification of Italian populations was based mainly on alleles similar to Yugoslav, with the difference concerning the variability level inside them, and genetic diversity of Italian populations was more pronounced on the basis of certain loci (Phi1, Adh1).

— The Portuguese populations were generally found to contain the same common alleles, but some new, rare alleles were also found.

— Compared to the French populations, the Portuguese populations were more variable, but also more closely related.

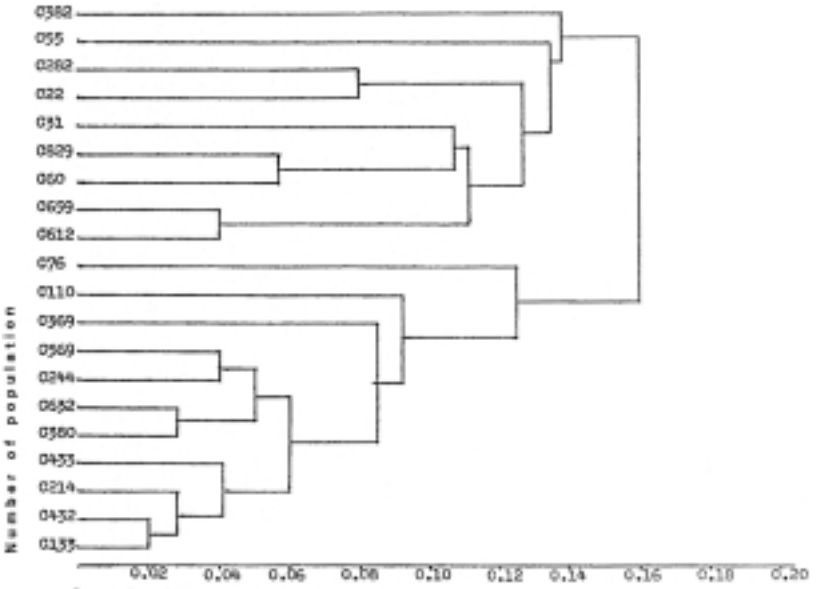

Fig. 1 - CLUSTER ANALYSIS OF POPULATION GROUP 17

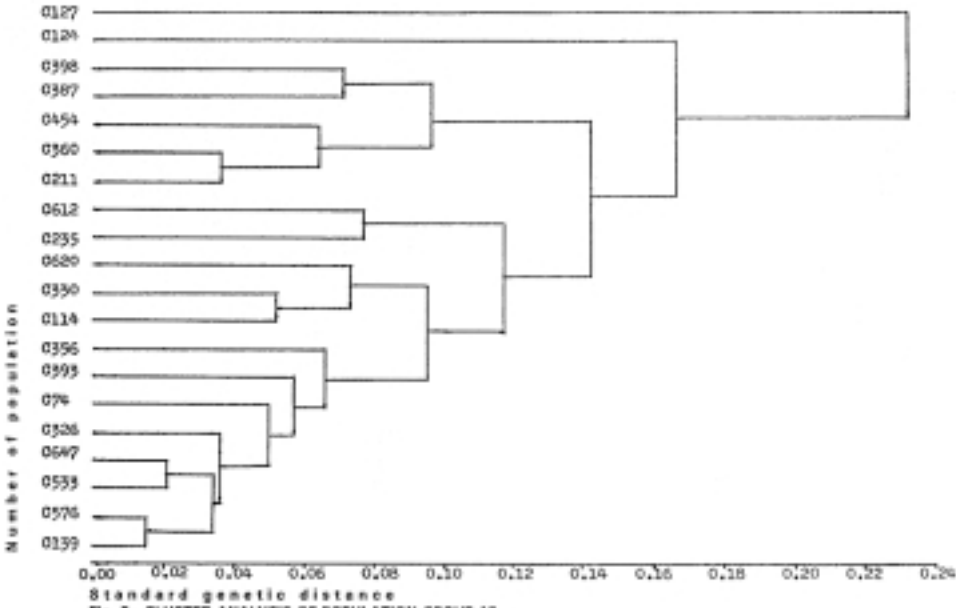

Fig. 2 - CLUSTER ANALYSIS OF POPULATION GROUP 18

A relatively low variability and an increased diversity in the French populations are possible due to an increased number of homozygous loci; even those loci which are usually polymorphic in open pollinated populations were homozygous in the French material.

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ГЕНЕТСКА ВАРИЈАБИЛНОСТ И ДИВЕРГЕНТНОСТ СЕЛЕКЦИОНОГ МАТЕРИЈАЛА КУКУРУЗА КОЈИ ПОТИЧЕ ОД ДОМАЋИХ И СТРАНИХ ПОПУЛАЦИЈА, ДЕТЕРМИНИСАНА НА БАЗИ БИОХЕМИЈСКО-ГЕНЕТСКИХ МАРКЕРА

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Резиме

Изоензими се користе као генетски маркери, а њихов број треба да буде довољно велик да би покривеност генома била што већа и као такви су маркери гена за тражена агрономска својства. Употребљени маркери су производи 21 мапираног гена, што представља релативно поуздан број за тумачење генетске основе одређених особина генотипа. Генетска варијабилност и дивергентност су значајне за популације и самооплодне линије, као основни материјал у селекцији и стварању нових хибрида кукуруза. Из тог разлога анализирано је неколико група популација кукуруза различитог порекла. Две групе југословенских популација, италијанска, португалска и француска колекција биле су оцењене на основу алелне варијабилности за 21 локус и стандардне генетске удаљености унутар сваке популације. Југословенске колекције кукуруза су показале високу хетерогеност на бази изоензима као ген. маркера. Генетска дивергентност италијанске колекције је наглашена на бази неких локуса, а португалска је имала више полиморфних и хетерозиготних локуса од француске колекције, што значи већи потенцијал генетске варијабилности. Интер-генетска варијабилност између популација и њихове географске локације веома су значајан услов у селекцији биљних врста и њихов хетерозис.