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PROTEIN POLYMORPHISM AND HETEROSIS OF MAIZE

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Rezumat. Lucrarea prezentată a fost concepută în scopul studierii posibilităţilor de evaluare moleculară a hibrizilor de porumb pe baza utilizării formelor moleculare a zeinei (FMZ). Analiza specificului polimorfismului zeinei permite de a constata efectul de heterozis pozitiv la 40 de combinaţii hibride de porumb, care se caracterizează printr-un nivel înalt de heterozis reproductiv în F_1. După FMZ s-a constat rolul deosebit al formei materne, în manifestarea expresiei genelor structurii endospermului în combinaţiile hibride. S-a propus folosirea la nivel de FMZ a valorilor heterozisului real după forma maternă (Hreal+) a fiecărui hibrid respectiv: pentru identificarea şi diferenţierea acţiunii genelor structurii endospermului de porumb (o2, su2, wx1); pentru alegerea formelor parentale în scopul creării hibrizilor competitivi de porumb; pentru a prognoza dificultăţile metodice posibile la aprecierea autenticităţii combinaţiilor hibride de porumb F_1.

Key words: Gene, Heterosis, Hybrid, Maize, Polymorphism, Protein, Zein.

INTRODUCTION

At present the study of protein polymorphism, as a marker of the genetic and breeding forms is one of the most important directions of biochemical genetics for agricultural plants and in particular for the maize culture (A. Sozinov, 1985; V. Konarev et al,1993; M. Zlokolica et al. (1993), S. Mladenovic-Drinic, K. Konstantinov, 1996; A. Rotari et al.,2004). This direction is elaborated intensely at the Department of Selection, Genetics and Biotechnology of the State Agrarian University of Moldova in collaboration with the laboratory of Maize and Sorghum Research Institute of Moldova (A. Palii, 2003; G. Comarova, 2002). The obtained valuable results show a wide spectrum of the efficient use of protein storage polymorphism - zein polymorphism (G. Comarova, 2002). So, for the genetic aspect it has been studied: the nature of zein molecular forms heredity (G. Comarova, 1998) and the potential possibilities of protein polymorphism for identification of direct pleiotropic effect of endosperm structure genes (A. Palii et al., 1995). It has been done a rather detailed study of zein polymorphism, for the selection aspect, in the following directions: the protein marker of homogeneously and typically degree of initial selected material of maize (G. Comarova et al., 1994; A. Rotari et al., 1996; G. Comarova et al., 2002); the identification of maternal forms in a mixture of hybrids and lines seeds of maize at the level of zein molecular forms (A. Rotari et al., 1996).

At the same time it is interesting to know the possibilities of zein molecular forms (ZMF) utilisation in determining the degree of heterosis effect, because heterosis is the general factor of a successful selection of highly productive maize hybrids with improved seed quality (G. Comarova et al., 1995). Therefore the aim of this research consisted in studying, at the level of ZMF: a) the character of heterosis effect revealing for different hybrid maize combinations; b) the possibilities of heterosis effect revealing for identification and differentiation of endosperm structure genes expression; c) the potential heterosis effect revealing for molecular passportization of registered maize hybrids.

MATERIALS AND METHODS

As research object were chosen two inbred maize lines A 632 and A 619; their mutant analogues with endosperm structure genes: opaque-2 (o2), sugary-2 (su2), waxy-1 (wx1); their hybrid combinations (direct and reverse); 21 registered maize hybrids of Moldovan breeding and their parental forms (45 inbred lines and 17 hybrid combinations).

Zein molecular forms were studied by the method of electrophoresis in polyacrylamide gel, acidic buffer, in the presence of urea (Standard Moldovean, SM 233 : 2003). The analysis of seed's chemical

content was conducted by Infrared Analyser Scanner, Model 4500. The degree and the type of heterosis expression in the studied hybrid combinations were analysed by the calculation method of heterosis effect criterion's determination: hypothetical heterosis (Hip) and real heterosis (Hreal).

RESULTS AND DISCUSSIONS

According to numerous experiences of selection works, the lines A632 and A619 possess a high combining ability and the hybrid combinations of these lines are characterised by a high degree of positive effect revealing the reproductive heterosis - according to the grain yield (tab.1). At the same time, in most of the cases it can be observed (without taking into consideration the dose of introduced endosperm structure genes) a negative or intermediate heterosis effect, by qualitative content of nitrogenic metabolites of maize seed (protein, lysine) which serves as leading criterion of qualitative maize selection (tab.1).

Table 1

The heterosis effect in mutation hybrid combinations (A632 x A619) and their normal analogues by grain yield and seed's chemical content

Genotype of hybrid combination	Hip (%)			Hreal (%)		
	Grain yield	Protein	Lysine	Grain yield	Protein	Lysine
norm x norm	+62	-6	-2	+58	-11	-3
norm x o2	+41	-15	+5	+36	-18	-15
norm x su2	+88	-2	+1	+58	-5	-9
norm x wx1	+63	-10	-5	+52	-11	-13
o2 x norm	+82	-14	-3	+61	-16	-23
su2 x norm	+167	-2	-12	+116	-19	-24
wx1 x norm	+104	+6	+7	+125	-1	+4
o2 x o2	+57	+1	-1	+50	+1	-2
su2 x su2	+162	-16	+8	+142	-12	-4
wx1 x wx1	+108	+2	-12	+99	+2	-17

On the contrary, a positive heterosis effect of zein molecular forms has been established for normal hybrid combination and its mutant analogues (o2, su2, wx1), independently of the corresponding gene introduced dose (tab.2, columns 8, 9).

The analyses of zein spectrum formulas, which were calculated on the basis of the obtained electrophoregramms and densitogramms indicate, that "hybrid" protein spectra, of the first filial generation (F_1) contain both maternal and paternal spectra (tab.2, columns 2-5). This fact confirms the postulate concerning the codominance character of protein electrophoretic components heredity.

But these zein spectrum formulas of the studied hybrid combinations don't reveal any differences between direct and indirect crosses of parental forms. This fact does not allow to define the concrete nature of endosperm structure genes expression in hybrid combinations at protein molecules level (tab.2, columns 4, 5).

It is known, that one of the most important conditions of grain hybridity determination (F_1) is the enrichment of EF zein spectrum of maternal form with peptide components, which is specific only for zein of paternal forms. That's why it has been done visual analyses of zein hybrid spectra by marking the protein system of paternal form.

Such a method allowed us to reveal the nature of endosperm structure genes expression in the studied reciprocal hybrid combinations. According to the table 2 (columns 6, 7) the genom's role of A619 line, as a paternal form is more important than the genom's role of A632 line, as a paternal form of studied hybrid combinations. For example, the number of ZMF of A619 line which enriched the "hybrid" EF zein profiles of mutant and normal hybrid combinations varies from 1 to 6 molecular forms, but for A632 line the limit of ZMF variation is narrower (from 2 to 3).

And besides, there are no differences concerning the number of ZMF in the direct and reverse hybrid combinations between normal inbred lines - A632 and A619. At the same time, the presence of

Table 2

The endosperm structure gene expression in the maize hybrid combinations according to the zein molecular forms (ZMF)

Genotype of hybrid combination	Total number of ZMF				Number of ZMF which enrich the "hybrid" EF zein profiles		Hreal (♀) by ZMF	
	♀	♂	F_1					
			direct	reverse	direct	reverse	direct	reverse
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
A632norm x A619norm	11	11	14	14	3	3	27	27
A632norm x A619o2	11	10	12	12	1	2	9	20
A632o2 x A619norm	8	11	14	14	6	3	75	27
A632o2 x A619o2	8	10	12	12	4	2	50	20
A632norm x A619su2	11	10	12	12	1	2	9	20
A632su2 x A619norm	9	11	14	14	5	3	56	27
A632su2 x A619su2	9	10	12	12	3	2	33	20
A632norm x A619wx1	11	10	12	12	1	2	9	20
A632wx1 x A619norm	9	11	14	14	5	3	56	27
A632wx1 x A619wx1	9	10	13	13	4	3	44	30

endosperm structure genes in hybrid combinations allows to reveal the quantitative differences between the ZMF markers of reciprocal combinations.

Also, it should be mentioned another fact: zein marker of paternal line A 619 is more strongly revealed on the maternal line A632 with endosperm structure genes. For example: the number of ZMF varies from 6 to 4 for mutant combinations with gene *o2*; from 5 to 3 for mutant combinations with gene *su2* and from 5 to 4 for mutant combinations with gene *wx1*. Therefore, it has been determined the differences in expression of *o2, su2* and *wx1* genes in those hybrid combinations where the maternal form was just the inbred line A632 with the presence in its genom of the corresponding endosperm structure gen.

The obtained results were considered like an essential argument for the expedient studying of the maternal role of genom's maternal form in revealing the expression of endosperm structure genes at the level of protein molecules according to the degree of hetrerosis effect. For such a purpose it has been calculated the value of real heterosis by ZMF, but with some modification. There was included the value of maternal form (but not the best value of one parental form) in the formula of real heterosis:

$$H\ real\ ♀ = \frac{F1 - P♀}{P♀} \times 100\ \%$$

The data shown in table 2 (columns 8, 9), indicate a high degree of positive heterosis effect for those hybrid combinations, whose maternal form is the inbred line A632, which carries one of the examined endosperm structure gene. For example: the real heterosis (by maternal form) for hybrid combinations with gene *o2* varies from 70% to 50%; for hybrid combinations with gene *su2* - from 56% to 33% and for hybrid combinations with gene *wx1* - from 56% to 44%.

Therefore, the detected differences of endosperm structure genes expression testify that the absolute values of the real heterosis by maternal form allow to differentiate the action of gene *o2* from the action of genes *su2* and *wx1*, which change the nitrogen and carbohydrate metablism accordingly.

The same method of calculation - that is the determination of real heterosis by maternal form - was used for studying the potential heterosis effect revealing for zein molecular forms passportization of registered maize hybrids of Moldovan breeding. It should be mentioned that this part of the investigations was based on the great volume of experiments, according to the study of zein polymorphism at 21 registered maize hybrids and 45 inbred lines, which showed their parental forms in different combinations.

According to the received results it was made up the catalogue of electrophoretic zein passports for service use. There are electrophoregramms, densitogramms and zein spectrum formula for every parental inbred line according to its hybrid combination and their classification into four groups of hybrids: single cross, single cross modification, three way cross and double cross. It is shown the presence or absence of protein markers for the hybridity determination of registered Moldovan maize hybrids in every electrophoretic passport.

The data, shown in table 3 indicate a positive effect of hypothetical heterosis by ZMF for all studied and registered hybrids with a high degree of reproductive heterosis: from 10% to 58% of Hip. At the same time, the value of real heterosis by maternal form (Hreal ♀) in comparison with hypothetical heterosis (H ip) enables us to do a more detailed study of the obtained results.

It has been established the tendency of predominance of real heterosis effect over the hypothetical one (H real ≥ H ip) as the characteristic for groups of single cross and double cross hybrids. At the same time it has been noticed the inversetendency for groups of modified single cross hybrids and three way cross hybrids, when the value of real heterosis by maternal form is decreasing in comparison with hypothetical heterosis (Hreal ≤ Hip).

Table 3

The heterosis effect in registered maize hybrids (F_1) of Moldovan breeding by zein moleculare forms

Single cross			Single cross modified		
Hybrid	Hip (%)	Hreal ♀ (%)	Hybrid	Hip (%)	Hreal ♀ (%)
Mold.291 MRf	10	22	Mold.205 ALCRf	36	12
Mold.349 KCRf	22	22	Mold.226 ACRf	15	7
Mold.424 MRf	24	44	Mold.381 ALCRf	58	50
Mold.450 MRf	17	27	Mold.421 MRf	27	56
Mold.456 MRf	22	27	Mold.425 MRf	41	33
Kish.307 LP	29	0	Porumb. 170ACRf	13	26
Δ	+18		Δ	-32	
Double cross			Three way cross		
Hybrid	H ip (%)	Hreal ♀ (%)	Hybrid	Hip (%)	Hreal ♀ (%)
Bemo 182 CRf	23	27	Bemo 160 MRf	15	15
Bermold 184 CRf	22	28	Bemo 181 CRf	20	9
Mold. 215 MRf	36	36	Mold. 257 CRf	31	24
Nemo 216 CRf	21	11	Mold. 411 MRf	18	8
Mold. 330 MRf	23	45			
Δ	+22		Δ	-28	

$\Delta = (H_{real} - H_{ip})$

The obtained data require a more detailed study at a molecular level that will allow us to make sense of the efficient utilisation at the revealing degree of real and hypothetical heterosis effect for maize selection.

CONCLUSIONS

· ZMF permit to reveal a positive heterosis effect in hybrid combinations with a high degree of reproductive heterosis

· ZMF characterise the decisive role of maternal form in revealing the endosperm structure genes expression in hybrid combinations

· ZMF allow using the value of Hreal+ (real heterosis by maternal form)...

... to identify and differentiate the genes o2, su2, wx1 ,

... to select the parental forms in order to create hybrid combinations with high productivity during a short period,

... to predict the possible methodological difficulties when appreciating the maize hybrid combination originality (F_1).

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