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PARAMETERS IN THE ESTIMATION OF THE MOST SUITABLE F_2 POPULATION SIZE IN CONVENTIONAL MAIZE (*Zea mays* L.) BREEDING PROGRAMS

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The objective of the present study was to observe differences among four sizes of the F_2 populations (100, 200, 300 and 500 plants) on the basis of test-crosses for grain yield according to the average values of the populations, genetic and phenotypic variances, genotypic and phenotypic coefficients of variations and broad-sense heritability. The values of genetic variance did not significantly differ over population sizes

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according to all possible comparisons, including the comparison of values obtained for the phenotypic variance. Furthermore, the values of broad-sense heritability (67.8%-69%) did not significantly vary over different F_2 population sizes. Genetic variability of the observed progenies, as a principal prerequisite of successful selection, was at the satisfactory level in all population sizes.

Key words: genetic variability, F_2 population, heritability, maize, recombination, yield

INTRODUCTION

The F_2 population consisting of two inbred lines is an initial material that is mostly exploited in the programmes of the maize (*Zea mays* L.) inbred lines development, WOLF *et al* (2000), BABIĆ *et al* (2008). Use of F_2 population is also very often in commercial inbred lines improvement for different traits, RADENOVIĆ *et al* (2007), MLADENOVIĆ DRINIĆ *et al (*2009). Complex of different factors influence recombination occurrences in F_2 population. The number of gene combination that differ from parents, depends on the number of genes in which parents (inbred lines) differ each other, gene linkage, as well as, the number of alleles that each gene locus has**.** Additionally, linkage reduces the frequency of gene recombination and practically it increases parental types in F_2 population. Another potential genetic consequence of hybridization concept is linkage drag (genes that occur in the same chromosome constitute a linkage block). Also, phenomenon of crossing over provides an opportunity for linked genes to be separated and not inherited together. Sometimes a number of genes are so tightly linked that they are resistant to the effects of recombination. If desired gene is strongly linked with not desired genes, a cross to transfer a desired gene will invariably be accompanied by the linked undesirable genes, MAREN (2007), ŽIVANOVIĆ *et al* (2007).

Also, different spectra of other factors, genetically and environmentally influenced could affect segregation ratio of different traits in maize, VANCETOVIC (2008). If parents differ only in 10 allelic genes, theoretically the number of different genotypes in the F_2 population is 59,049. Such great numbers of recombinants practically cannot be sown in the breeding fields, especially as breeding programs include a great number of F_2 populations. The size of F2 population and the number of the F_2 population, as well as, a good balance between these two values define to a great extent the success of the breeding programmes, DELIĆ *et al* (2005). The aim of this study was to determine the optimum, lowest number of plants that represents the F_2 population, as well as, the change in genetic parameters: genotypic and phenotypic variances, heritability, genotypic and phenotypic coefficients of variations in relation to the change of the population size. A relatively lower number of plants in the F_2 generation provide an easier phenotypic estimation of a progeny, a greater number of observed combinations, as well as, the reduction of material costs necessary for the process of breeding.

MATERIAL AND METHODS

The F_2 population obtained out of two inbred lines, L500 and L21-92, was used in the present study. Inbred line L500 is a dent type, of a medium late maturity (FAO 550) and with a good combining ability with the inbred of the *Lancaster* origin. Inbred line L21-92 is a semi-dent type, of the FAO maturity group 700 showing combining abilities similar to the inbred L500. In 2003, 1600 S_0 plants of the F_2 population (L500xL-21) was self-pollinated and simultaneously crossed to a tester, an inbred line R25. The dent inbred line R25 of a medium maturity (FAO 450) combines well with both, L500 and L21-92. After harvest, 500 test hybrids out of 720 successful crosses were selected and studied in the succeeding two years in the field trial set up according to the *Nested* design (incomplete block design, *random model*, COCHRAM and COX, 1957; HALLAUER and MIRANDA (1988), with replications within sets. In 2006, the 3-replication trial with 20 tests hybrids within each of 25 sets was set up in four locations. The same method was followed in 2007, when the trial was set up in three locations. The hybrid L92 x L 1325 was sown within each set as a check. The size of the one-row elementary plot with 20 plants and sowing density of 62,112 plants ha^{-1} amounted to 3.22 m^2. Sowing and harvesting were done by hand. The grain yield was estimated in t ha^{-1} and computed to 14% grain moisture. Biometric data processing was done for the sizes of the F_2 population of 100, 200, 300, and 500 plants. The combinations of 100, 200 and 300 test hybrids were obtained by a computer simulation. All investigated combinations were obtained over the sets; hence the total number of combinations amounted to:

Thirty samples for each population size (100, 200 and 300) were randomly selected from the basic population size of 500 test hybrids. The analysis of variance in the *Nested* design - *random model* (COCHRAM and COX, 1957; HALLAUER and MIRANDA, 1988), (Table 1) was performed for both, the F_2 population size of 500 test hybrids and for each sample (of 30 samples) for the F_2 population sizes of 100, 200 and 300 test hybrids. Mean squares were used to estimate genetic variance (σ_g^2), standard error of genetic variance ($SE\sigma_g^2$), phenotypic variance (σ_f^2), standard error of phenotypic variance, coefficient of genetic variation (CV_g), coefficient of phenotypic variation (CV_f), broad-sense heritability (h^2) and standard error of heritability (SEh^2) for the same population sizes.

Genetic variance (σ_g^2), according the ANOVA parameters (Table 1):

$$\sigma_g^2 = \frac{MS_5 - MS_6}{r \times l}$$

According to values of two standard errors of genetic variance ($2SE\sigma_g^2$), significance of difference from zero of estimated genetic variances (σ_g^2) was tested, FALCONER (1960 and 1981).

$$SE\sigma_g^2 = \sqrt{\frac{2}{(r \cdot l)}\left[\frac{(MS_5)^2}{s(f-1)+2} + \frac{(MS_6)^2}{s(f-1)(l-1)+2}\right]}$$

where: f - number of test hybrids within a set
s - number of sets
l - number of locations

Coefficient of genetic variation (CV_g), %:

$$CV_g = \frac{\sqrt{\sigma_g^2}}{\overline{X}} \cdot 100$$

Phenotypic variance (σ_f^2):

$$s_f^2 = s_g^2 + \frac{s_{gl}^2}{l} + \frac{s_e^2}{r \times l} = \frac{MS_5 - MS_6}{r \times l} + \frac{MS_6 - MS_7}{r \times l} + \frac{MS_7}{r \times l}$$

Coefficient of phenotypic variation (CV_f), %:

$$CV_f = \frac{\sqrt{\sigma_f^2}}{\overline{X}} \cdot 100$$

Broad-sense heritability (h^2), %: $h^2 = \frac{\sigma_g^2}{\sigma_f^2}$ x 100

Table 1.*ANOVA in the Nested design and expected mean squares (random model) (*COCHRAM *and* COX, *1957;* HALLAUER *and* **MIRANDA**, *1988)*

Var. source	df	Mean square	Expected mean square	F value
Location	l-1	MS_1	$\sigma^2_e + r\sigma^2_{fl/s} + f\sigma^2_{r/s/l} + rf\sigma^2_{ls} + rfs\sigma^2_l$	MS_1/MS_3
Set	s-1	MS_2	$\sigma^2_e + r\sigma^2_{fl/s} + rl\sigma^2_{f/s} + f\sigma^2_{r/s/l} + rf\sigma^2_{ls} + rfs\sigma^2_l$	MS_2+MS_5/MS_3+MS_6
Loc.x set	(l-1) (s-1)	MS_3	$\sigma^2_e + r\sigma^2_{fl/s} + f\sigma^2_{f/s/l} + rf\sigma^2_{ls}$	$MS_3/MS6$
Rep./set./loc.	ls (r-1)	MS_4	$\sigma^2_e + f\sigma^2_{r/s/l}$	MS_4/MS_7
Hyb./set	s (f-1)	MS_5	$\sigma^2_e + r\sigma^2_{fl/s} + rl\sigma^2_{fs}$	$MS_5/MS6$
Hyb.x loc./set	s (f-1) (l-1)	MS_6	$\sigma^2_e + r\sigma^2_{fl/s}$	MS_6/MS_7
Error	ls (r-1) (f-1)	MS_7	σ^2_e	
Total	rslf-1			

RESULTS

Highly significant estimates of mean squares for the test hybrids within tests with 30 samples within each test for the population sizes of 100, 200 and 300 tests hybrids were obtained by the analysis of variance in the *Nested* design. Mean squares for hybrids within sets for the basic sample of 500 test hybrids were also highly significant (Table 2).

Table 2. Mean squares from the analysis of variance in the Nested design for the population size of 500 test hybrids

Source of variation	DF	MS
Location (L)	5	11632.672**
Set (S)	24	51.824
L x S	120	53.834**
Replication /S/L	300	4.450**
hybrid/S	475	7.071**
Hybrid x L/S	2375	2.296**
Error	5700	1.687

* $p < 0.05$,** $p < 0.01$

Estimated values of genetic variance (σ_g^2) in all four studied population sizes were significant, as they were two or a few times higher than the value of the corresponding standard error ($SE\sigma_g^2$), (FALCONER, 1960, 1981). A difference between minimum and maximum values of genetic variance over samples within a population size was greater in the populations with a lower number of test hybrids.

The estimates of standard error of genetic variance ($SE\sigma_g^2$) decreased over the increasing number of test hybrid`s, hence the lowest estimate was recorded in the population size of 500 test hybrids (Table 3). A relative parameter of genetic variation, the coefficient of genetic variation (Cv_g), showed the similar trend with the increasing size of the population as in case of the estimates of genetic variance.

Table 3. Components of variance with the corresponding standard errors, coefficients of variation and heritability in the population size of 100, 200, 300 and 500 tests hybrids respect

	$\bar{X}$ t/ha	σ_g^2	CV_g(%)	σ_f^2	CV_f (%)	h^2(%)	$SE\sigma_g^2$	$SE\sigma_f^2$	SEh^2
Population size 100 plants									
min.	9.14	0.154	4.05	0.277	5.36	53.90	0.040	0.041	0.145
max.	9.81	0.465	7.27	0.623	8.42	74.70	0.090	0.091	0.147
mean	9.53	0.285	5.56	0.416	6.73	67.80	0.060	0.061	0.145
Population size 200 plants									
min.	9.38	0.191	4.50	0.307	5.72	61.20	0.032	0.033	0.103
max.	9.70	0.378	6.43	0.508	7.51	74.40	0.052	0.053	0.104
mean	9.56	0.272	5.44	0.399	6.59	68.00	0.041	0.042	0.103
Population size 300 plants									
min.	9.41	0.197	4.61	0.318	5.87	61.90	0.027	0.028	0.084
max.	9.66	0.324	6.02	0.463	7.19	71.90	0.039	0.039	0.085
mean	9.54	0.275	5.48	0.403	6.64	68.00	0.034	0.034	0.084
Population size 500 plants									
mean	9.56	0.284*	5.58	0.412*	6.71	69.00*	0.027	0.027	0.066

* , > 2SE , respectively

The average values of the coefficient of genetic variance ranged from 5.44% in the population of 200 test hybrids to 5.58 % in the population of 500 test hybrids. The determined values of phenotypic variance ranged from 0.399 in the population of 200 test hybrids to 0.416 in the population of 100 test hybrids and were higher than the values of genetic variance, considering that they also included environmental variations. The greater size of the population was, the lower value of standard error of phenotypic variance was. The same trend was observed for the values of standard errors of heritability (Table 3).

The calculated values of heritability were quite uniform and ranged from 67.8% in the population of 100 test hybrids to 69 % in the population of 500 test hybrids, provided that the average values of heritability in the populations of 200 and 300 test hybrids were identical and amounted to 68%.

The six possible comparisons of average values of genetic variances of the studied population sizes did not show significant differences (Table 4). Furthermore, average values of phenotypic variances of the studied population sizes were compared in the same way in all possible combinations, and not a single comparison showed significant differences (Table 5). If the values of genetic and phenotypic variances are considered the criteria for a selection of a F_2 population size, the obtained results suggests that it is possible to work with 100 plants per a population without greater risk of loss of favorable genetic variation for grain yield.

Table 4. Genetic variances σ^2_g *(on the diagonal), differences among variances (above the diagonal) and* $\sigma^2_{g\ higher}/\sigma^2_{g\ lower}$ *ratio (below the diagonal)*

Population size	100	200	300	500
100	**0.285**	0.013	0.010	0.001
200	1.048	**0.272**	0.003	0.012
300	1.036	1.011	**0.275**	0.009
500	1.004	1.044	1.033	**0.284**

Table 5. Phenotypic variances σ^2_f *(on the diagonal), differences among variances (above the diagonal) and* $\sigma^2_{f\ higher}/\sigma^2_{f\ lower}$ *ratio (below the diagonal)*

Population size	100	200	300	500
100	**0.416**	0.017	0.013	0.004
200	1.043	**0.399**	0.004	0.013
300	1.032	1.010	**0.403**	0.009
500	1.010	1.032	1.022	**0.412**

DISCUSSION

The difference between minimum and maximum values of genetic variance over samples within the population size was greater in the populations with a lower number of test hybrids, while the values of standard error of genetic variance decreased with the increase of the population size. These results are absolutely in agreement with results obtained by MIŠEVIĆ (1988) on each of three observed F_2 populations. Differences between average values of genetic and phenotypic variances among observed population sizes were not significant (Table 4). Estimated values of genetic variances for such a type of progenies (HS) were higher than those obtained by DRINIĆ (1995), and somewhat lower than those determined by ZANNONI and DUDLEY (1989) in studies of *test-crosses* of plants of F_2 populations of inbreeds genetically related to elite opposite testers. The coefficient of genetic variation ranged over a narrow interval (5.44 % - 5.58 %), as had been expected, considering that narrow intervals of variations and insignificant differences among population sizes were determined for genetic variance. If the comparison of the material in the process of selection is done on the basis of the values of test hybrids (*test-crosses*), as it was a case in the present study, it is important to consider effects of different types of testers and the generations of tested progenies on genetic parameters, primarily on the values of genetic variance. RAWLINGS and THOMPSON (1962) established that the total genetic variance of *test-crosses* had been determined by the gene frequency of testers and the level of dominance. If there is no dominance, genetic variance of *test-crosses* is equal for each of gene frequencies of testers. If the gene frequency of a tester r = 0.5, genetic variance of *test-crosses* is equal for all levels of dominance. These authors concluded that testers with a low frequency of desirable genes had an advantage over other testers in the rank of partial to complete dominance, considering that the decrease in the level of dominance led to the increase in genetic variance of studied *test-crosses*, hence the differentiation of observed progenies was more obvious and more credible, which was the principal objective of testing. Similar conclusions were drawn by ALISON and CURNOW (1966), who gave the advantage to testers of poorer values *per se*. Moreover, HALLAUER and LOPEZ-PEREZ (1979) stated that *test-crosses* of the same sets of S_8 inbreeds of the *BSSS* background expressed two-fold greater genetic variance when testers of a broad genetic base or testers of performance *per se* poorer than elite testers B73 (related) and Mo17 (unrelated) had been used. The present study included S_0 plants tested with the elite tester R25, which was the logical choice, considering that it was pedigree selection from the F_2 population of genetically close inbreeds L21-92 and L500, which cross to selected tester expressed a good combining ability. It assumes that selected recombinants would maintain high combining ability with the elite tester R25 in the subsequent selfing generations.

Number of plants influence accuracy of constructing of genetic maps in F_2 populations, which are of great importance for detailed genetic analysis and breeding of qualitative and quantitative traits. A total of 200 individuals were considered as adequate minimal number to construct reasonably accurate genetic maps, FERREIRA *et al* (2006).

Further investigations concerning number of plants which are sufficient to provide satisfied portion of variability in F_2 population obtained out of genetically similar inbred lines of maize (or inbred lines from the same heterotic group) should be tested over greater number of F_2 mapping populations for different traits. On this way should be more certain to estimate what scope of genetic variability is usually covered by different number of F_2 plants in conventional breeding programs which exploits narrow based population as breeding source materials.

Further use of molecular markers in F_2 recombination study should enable more precise reviling of genetic distortion and segregation and become promising applicable tool in breeding programs, YI CHUAN XUE BAO *et al* (2003), ZHAO BING *et al* (2006).

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PARAMETRI U OCENI ODGOVARAJUČE VELIČINE F2 POPULACIJE U KONVENCIONALNIM PROGRAMIMA OPLEMENJIVANJA KUKURUZA (*Zea mays L.*)

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I z v o d

U radu su ispitivane razlike između četiri veličine F_2 populacije od 100, 200, 300 i 500 biljaka na bazi test-ukrštanja, za prinos zrna, prema prosečnim vrednostima populacija, genetičkim i fenotipskim varijansama koeficijentima genetičke i fenotipske varijacije i vrednostima heritabilnosti u širem smislu. Vrednosti genetičke varijanse, nisu se značajno razlikovale između različitih veličina populacije u svim moguæim upoređenjima, što važi i za upoređenja dobijenih vrednosti fenotipske varijanse. Takođe, vrednosti heritabilnosti u širem smislu (67.8% do 69%) nisu značajno varirale sa promenom veličine F_2 populacije. Genetička varijabilnost ispitivanih potomstava, kao glavni preduslov uspešne selekcije, je bila na zadovoljavajućem nivou u svim veličinama populacije.

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