Full Length Research Paper

Selection and breeding experiments at the haploid level in maize (*Zea mays* L.)

Rotarenco Valeriu[1,2]*, Dicu Georgeta[1], Mihailov Mihail[2] and State Daniel[3]

[1]Procera Genetics, Muncii 47, Fundulea, Calarasi, 915200, Romania.
[2]Institute of Genetics and Physiology of Plants, Padurii 20, Chisinau, MD-2002, Republic of Moldova.
[3]Procera Agrochemicals Ltd, Muncii 47, Fundulea, 915200, Calarasi, Romania.

Selection at the haploid level allows increasing the efficiency of recurrent selection procedures. After four cycles of a recurrent selection approach involving haploid plants, grain yield of a synthetic population, SPC$_4$, was at the same level as that of current commercial hybrids. The SPC$_4$ population had a good performance in comparison with 27 synthetic populations obtained from Iowa State University. Transgressive segregation for plant height and ear length was revealed at the haploid level in two single crosses. It was demonstrated that the utilization of haploid plants to identify, create and improve elite breeding material might be very efficient.

Key words: *Zea mays*, haploids, recurrent selection, synthetic populations, hybrids, transgressive segregation.

INTRODUCTION

Within the last decade, the *in vivo* haploid induction technology has widely been used in maize breeding and research. In order to apply this technology, two main problems had to be solved: (1) producing haploids in large-numbers and (2) doubling the haploid chromosome set. After the creation of high-efficient haploid inducers, the production of maternal haploids became a routine procedure in maize (Coe, 1959; Tyrnov and Zavalishina, 1984; Lashermes and Beckert, 1988; Sarkar et al., 1994; Shatskaya et al., 1994; Chalyk, 1999; Röber et al., 2005; Rotarenco et al., 2010). However, the applied techniques of chromosome doubling still have some disadvantages namely; (i) plant survival might significantly be reduced, (ii) partially doubled haploids (DHs), mixaploids, with both poor pollen production and kernel yield are obtained and, as a result, an additional season to multiply the seeds of DHs is required. Besides, an experienced staff and appropriate facilities are needed to apply the DH technology successfully. Thus, using DH lines in breeding work has considerable difficulties so far.

Alternatively to the DH technology, pure haploid plants can be used for some breeding and research purposes. This is possible due to their ability to produce normal kernels after pollination with the pollen from diploid plants. Female fertility of haploid plants was studied by hand-pollinating them with the pollen from diploid inbred lines, the cross resulting in almost all of the ears carrying kernels. On the average, 27.4 kernels per ear of haploid plant were obtained in the first year of study and 26.3 in the second one (Chalyk, 1994). Geiger et al. (2006) estimated female fertility of haploid plants obtained by open-pollination with diploids in three locations. In their study, all haploids derived from more than 150 genotypes showed a certain degree of female fertility.

It was shown that the phenotype of maternal haploids corresponds to that of their diploid counterparts (Chase, 1964; Chalyk and Ostrovsky, 1993), and a population of haploid plants represents a random segregating population (Lashermes et al., 1988; Chalyk and Chebotar, 2000). Those studies allowed us to assume that selection for some agronomic traits and a genetic analysis at the haploid level can be efficient.

Haploid plants have already successfully been used for a genetic analysis of different quantitative traits - plant height, ear length and the number of kernel rows (Chebotar and Chalyk, 1996; Chalyk and Chebotar, 1999).

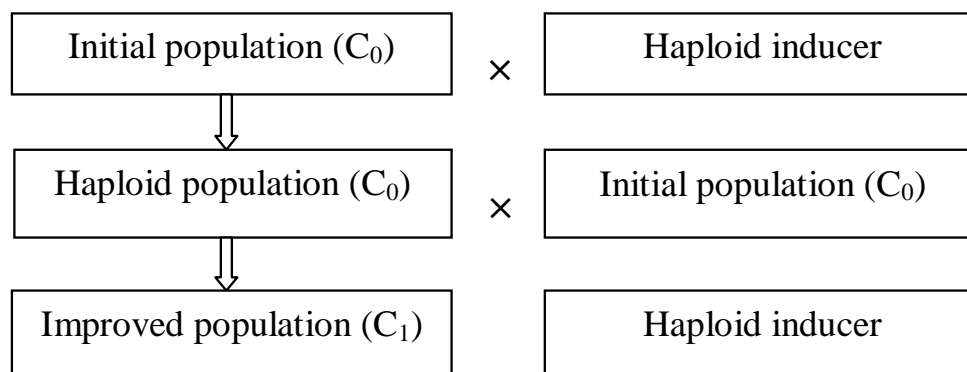*Corresponding author. E-mail: rotarenco@mail.md.

Figure 1. Schematic representation of the first cycles of haploid recurrent selection.

Synthetic populations are frequently used as source material to create inbred lines in maize breeding. To increase the frequency of elite-line production, synthetic populations need to be improved in connection to the goals of the breeding work. One of the methods for improving synthetic (heterogeneous) populations is recurrent selection (Sprague and Eberhart, 1977). An average gain per cycle for grain yield of the all known methods of recurrent selection ranged from 2 to 4% (Hallauer and Mirinda, 1988).

A recurrent selection scheme involving haploid plants, Haploid Recurrent Selection (HRS), was applied to improve two synthetic populations, SP and SA, for *per se* performance. It was revealed and described in our previous papers as a significant efficiency of this kind of recurrent selection resulting in a grain-yield increase per cycle of more than 10% (Chalyk and Rotarenco, 1999, 2001; Rotarenco et al., 2004).

In haploid and DH plants, allelic gene interaction (dominance and overdominance) is lacking that considerably facilitates selection for additive gene effects (Chase, 1952; Griffing, 1975) leading to the improvement of population *per se* (Hallauer and Miranda, 1988). In our opinion, haploid plants can also be useful for the identification of favorable germplasm. A comparative analysis of different populations at the haploid level would give us information about their value as a source of favorable genes with additive effects. The hybridization of populations can lead to transgressive segregation and that effect can easily be found among haploid plants.

The goals of our work were: (1) to evaluate the improved populations SPC_4 and SAC_3 in comparison with current commercial hybrids and the collection of populations from Iowa State University; (2) to identify favorable breeding germplasm using haploid plants; (3) to simulate the estimation of transgressive segregation at the haploid level in two single crosses.

MATERIALS AND METHODS

The SP population (FAO-330) was created from inbred lines (P346C, ms1334, Co125, MK01) and an open-pollinated variety - English Early (Iodent); its pedigree is: [(P346C × English Early) × (ms1334 × MK01y)] × (Co125 × MK01y). The SA population (FAO-295) was created from inbred lines N384S, P092, RV, and MR159 (Lancaster); its pedigree is: (N384M × P092) × [P092 × (Rf7 × MK159)].

Haploids were induced by open pollination at an isolated plot. Haploid inducer MHI (Chalyk, 1999) was used as a pollinator. The MHI inducer is homozygous for the *R1-nj* marker gene (Nanda and Chase, 1966) allowing haploids to be screened at the stage of dry seeds. This gene leads to a purple scutellum and a "purple crown" of the aleurone of F_1 kernels when unpigmented donors are pollinated by the inducer. Kernels with a haploid embryo and a regular triploid endosperm display an uncolored embryo and a red crown, whereas, F_1 kernels show pigmentation of both embryo and endosperm (Geiger and Gordillo, 2009).

Four and three cycles of HRS have been carried out in populations SP and SA respectively. From 1500 to 2000 haploid plants were cultivated for each selection cycle. About 300 visually selected plants were pollinated with a pollen mixture sampled from the diploid parent population. Seeds from about 50 ears (selection intensity of about 15%) with the highest values of ear length and the number of kernel rows were bulked for conducting the next cycle of HRS.

Schematic representation of the first cycles of haploid recurrent selection

The schematic representation of the first cycles of haploid recurrent selection is shown in Figure 1. The steps involved are as follows:

1. Induction of haploids in the C_0 population.
2. Pollination of selected haploids with a pollen mixture from diploid C_0 population.
3. Induction of haploids in the C_1 population to conduct the second cycle of HRS.

Two current commercial hybrids, Moldavian 291MV (FAO-290) and Porumbeni 331MV (FAO – 330) were used as checks.

In 2009, 27 populations from Iowa State University (FAO-500-700), 10 populations – B-SSS (P1, P9-17) 14 – N-SSS (P2, P6-8, P18-27) 1 – Iowa Early Rootworm Synthetic (P3), 1 – Lancaster Composite (P4), 1 – (Tuxpeno × Lancaster 2) Synthetic (P5) were tested and compared with our breeding material including the SPC_4 and SAC_3 populations. The estimation has been carried out at both the diploid and haploid levels.

Three breeding lines, MK01, 092, A619, and two F_1 hybrids between them, MK01 × A619 and MK01 × 092, were used to determine

Table 1. Means of two diploid populations improved by HRS (SPC4 and SAC3), a synthetic composed of these populations, and two check hybrids (2008, 2009).

Genotype	Grain yield (Mg ha^{-1})		Ear length (cm)		Kernel rows number		Plant height (cm)		1000 kernel weight (g)		Protein (%)		Oil (%)	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
SPC$_4$	9.7	10.3	18.0	20.3	17.7	18.1	298	300	310	299	10.9	11.6	4.7	4.7
SAC$_3$	5.1	5.2	15.7	16.4	13.7	13.4	212	221	244	247	12.0	11.8	5.3	5.0
SP C$_4$ × SA C$_3$	9.8	9.9	18.0	19.6	16.9	17.1	309^	310^	316	292	10.5	11.1	5.0	5.0
Moldavian 291MV	10.1	10.9	19.2	20.5	15.0*	16.2*	297	299	340	310	9.9	10.5	4.4	4.9
Porumbeni 331MV	9.8	9.9	18.1	18.4	14.5*	16.6*	260*	260*	350	315	10.2	10.4	4.4	4.7
LSD $_{(0.05)}$	0.9	1.1	2.7	2.9	0.8	1.2	9	10						

*Significantly (P<0.05) inferior to the SPC$_4$ population; ^ significantly (P<0.05) exceed the SPC$_4$ population.

the range of phenotypic variation at the haploid level. To show the range, samples have been divided into five equal groups (quintile), according to the rule of $n = log_2 N$ (n - number of groups, N - size of sample), and on the basis of the means of the groups, diagrams have been made.

Field tests were conducted in three replications on two-row plots of 10 m^2 size (row length: 7 m, distance between rows: 0.7 m) with plant density 60 plants per plot. Plant height was measured to top of the tassel. In diploids, after drying to moisture content of 14%, grain yield was determined. To estimate the weight of 1000 kernels, two 500-kernel samples were taken from the bulked seeds of each plot. The content of protein and oil was identified by Infratec ^{TM} 1241 Grain Analyzer.

RESULTS AND DISCUSSION

Population SPC$_4$ was significantly better performing than population SAC$_3$ for grain yield and growth traits (Table 1). As mentioned previously, the populations were created from different germplasm and that most likely, led to a different response to HRS. Our primary purpose was to obtain contrasting populations which would allow obtaining lines with a good combining ability in interpopulation crosses. However, the synthetic obtained from the hybridization of the populations, SPC$_4$ × SAC$_3$, displayed no superiority over the SPC$_4$ population for grain yield, whereas, for plant height, there was a significant increase (heterosis) (Table 1).

In local conditions, a grain yield close to 10 Mg ha^{-1} is usual for commercial hybrids with FAO up to 400. The grain yield of the synthetic population SPC$_4$ was 9.7 Mg ha^{-1} in 2008 and 10.3 Mg ha^{-1} in 2009. In our experiments, the check hybrids, Moldavian 291MV and Porumbeni 331MV, did not show a significant superiority over population SPC$_4$ for grain yield. For kernel-row number, the SPC$_4$ population significantly exceeded the checks, as well as for plant height over the hybrid Porumbeni 331MV. Kernel size (weight of 1,000 kernels) was bigger in the check hybrids, but almost in all cases the populations had a better performance for protein and oil content (Table 1).

Four populations from Iowa State University, P4, P11, P15 and P22, significantly exceeded the population SPC$_4$ for grain yield, three of them, P15, P17 and P18, had longer ears and none of them significantly beat the SPC$_4$ population for kernel row number (Figures 2, 3 and 4). Only one population, P23, was superior to population SPC$_4$ for plant height and ear length at the haploid level (Figures 5 and 6).

Good performance of the SPC4 population in comparison with commercial hybrids and the collection of populations from Iowa State University allowed us to conclude that HRS has a high potential for the improvement of synthetic populations *per se*. Comparable analysis of populations carried out at the haploid level showed that the improvement of the SPC$_4$ population indeed occurred due to the increase of the frequency of favorable genes with additive effects. Additionally, this experiment allowed characterizing three new populations, P18, P22 and P23, as a possible source of favorable genes with additive effects. In 2010, these populations were crossed with our breeding material with the purpose of using new germplasm for further improvement of populations. At the same time, we are expecting transgressive segregation in those crosses. We propose to use haploid plants to identify transgressive segregation. The simulation of this kind of estimation was rather clear and effective. About 15% of haploids derived from the hybrids, MK01 × 092 and MK01 × A619, significantly exceeded the haploids of the best parent lines for plant height and ear length (Figures 7, 8, 9 and 10). In the crosses of different populations, favorable-gene combinations can be fixed in the breeding material upon pollinating haploids with the pollen from diploids.
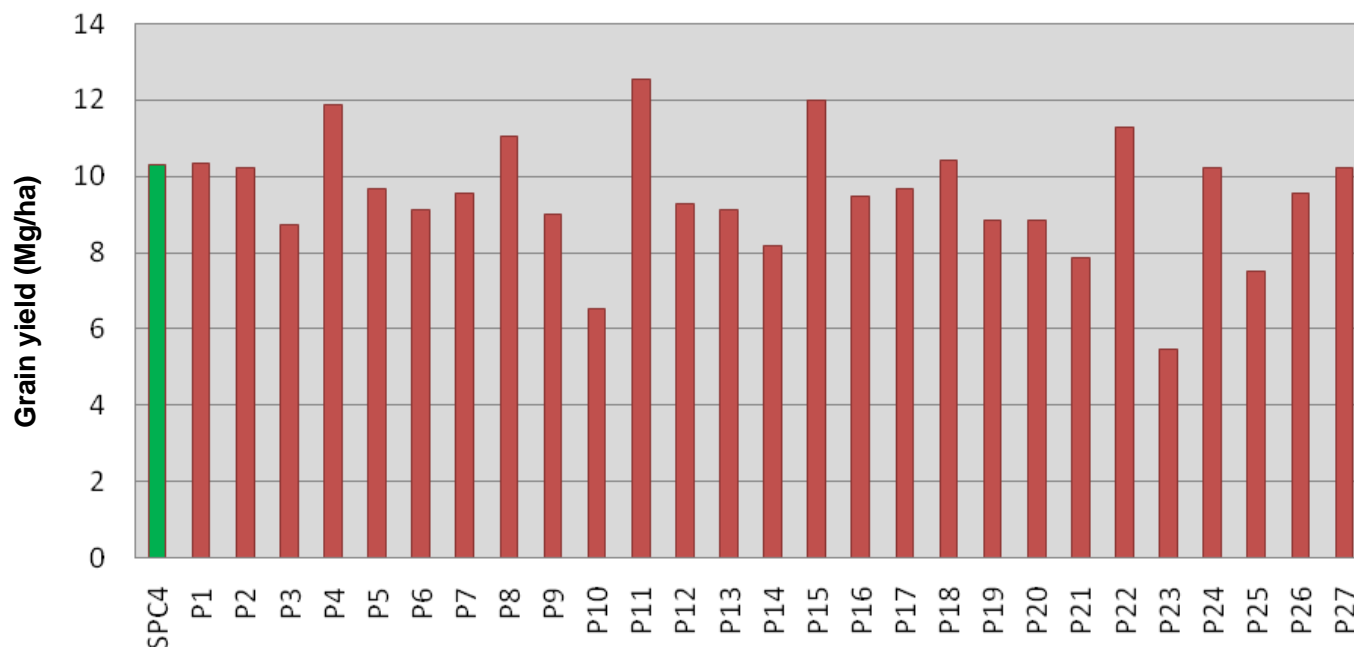
Figure 2. Distribution of the means of 27 synthetics from Iowa State University in comparison with SPC$_4$ for grain yield (2009).
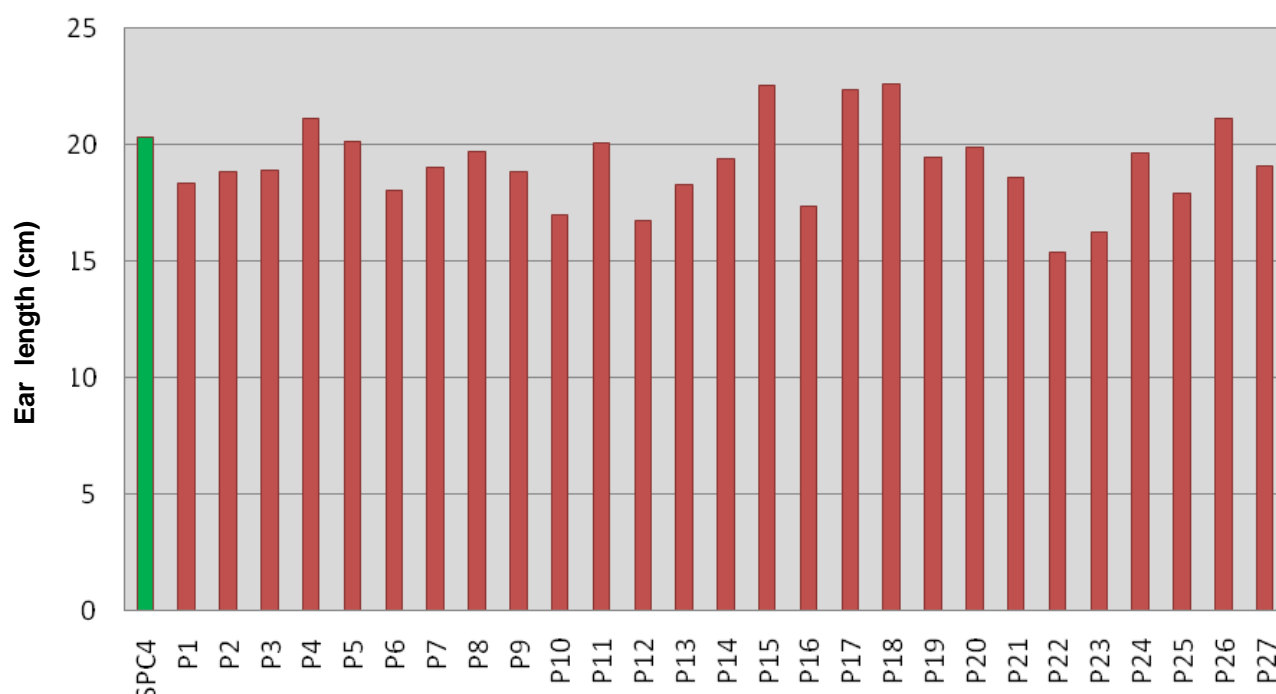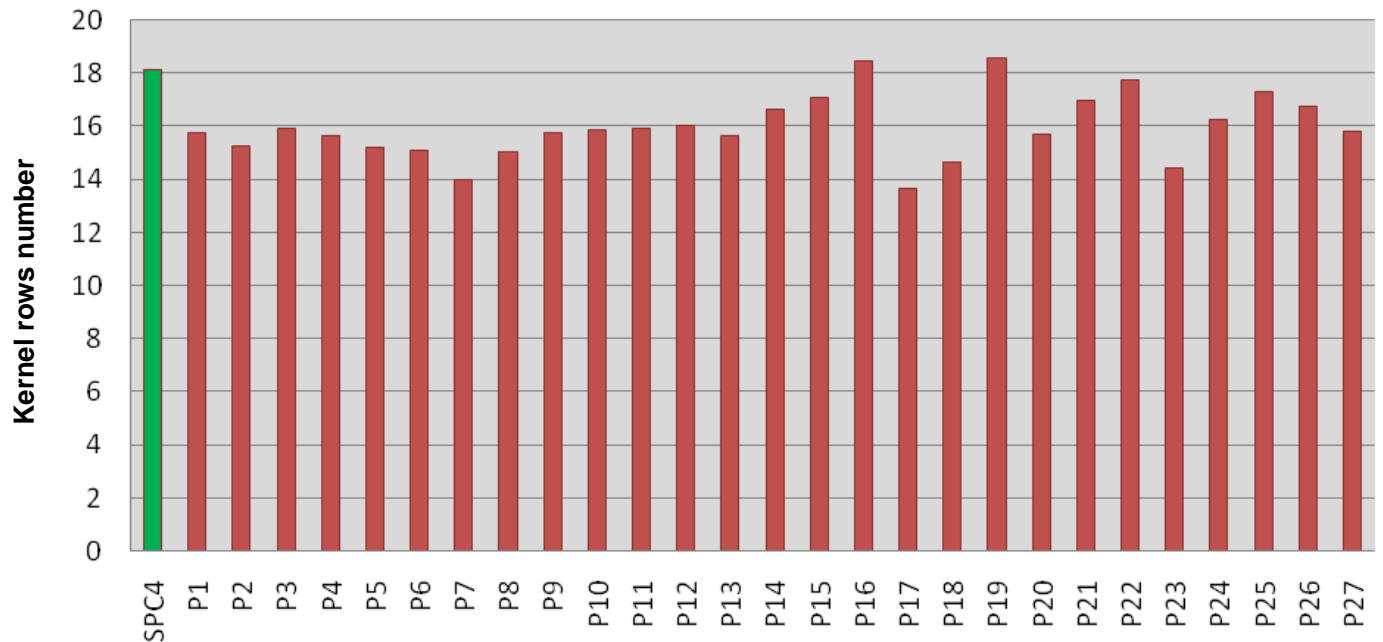

Figure 3. Distribution of the means of 27 synthetics from Iowa State University in comparison with SPC$_4$ for ear length (2009).

Conclusions

HRS significantly improved the vigor of synthetic populations. High-yield populations can be used not just as an initial material in breeding work, but also in maize production along or even instead of F$_1$ hybrids. Using haploids has a great potential for identifying valuable germplasm and accumulating genes with favorable additive effects in the breeding material. Haploidy requires more attention from researchers as a very convenient tool

Figure 4. Distribution of the means of 27 synthetics from Iowa State University in comparison with SPC_4 for kernel row number (2009).
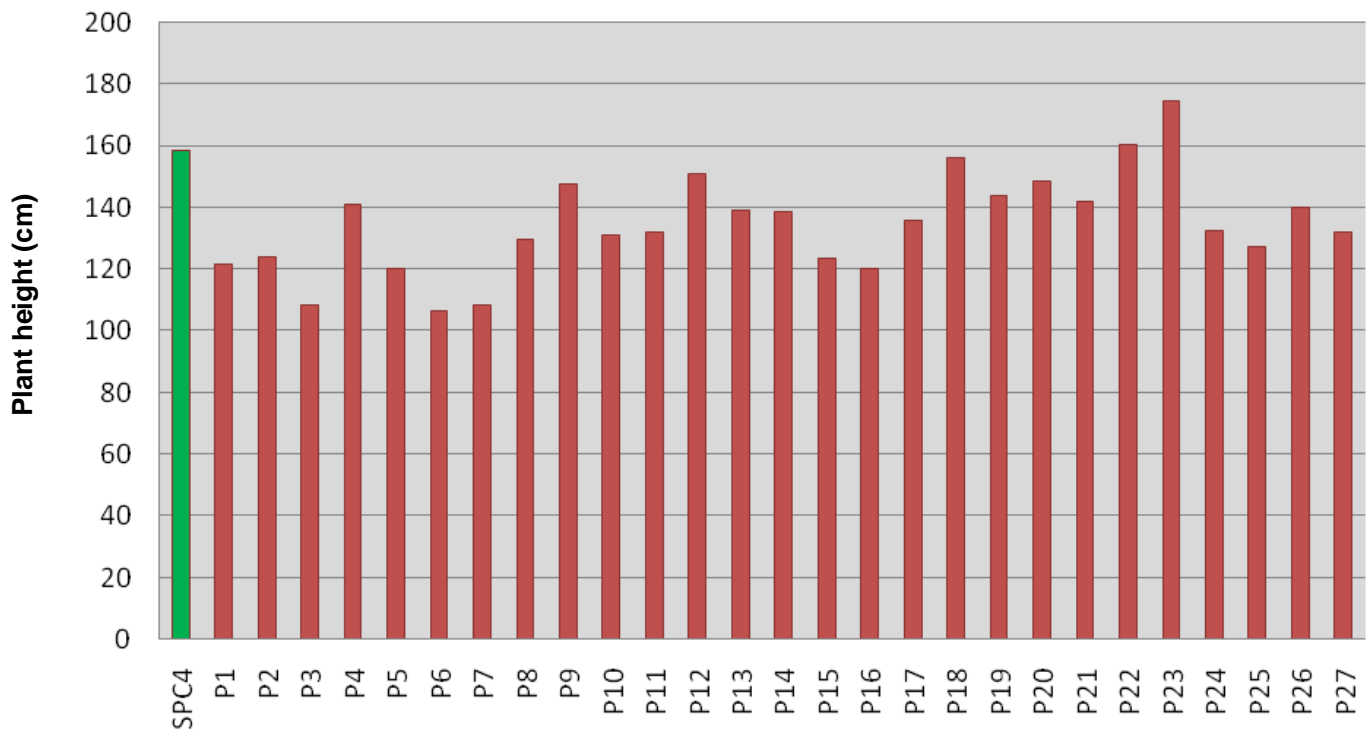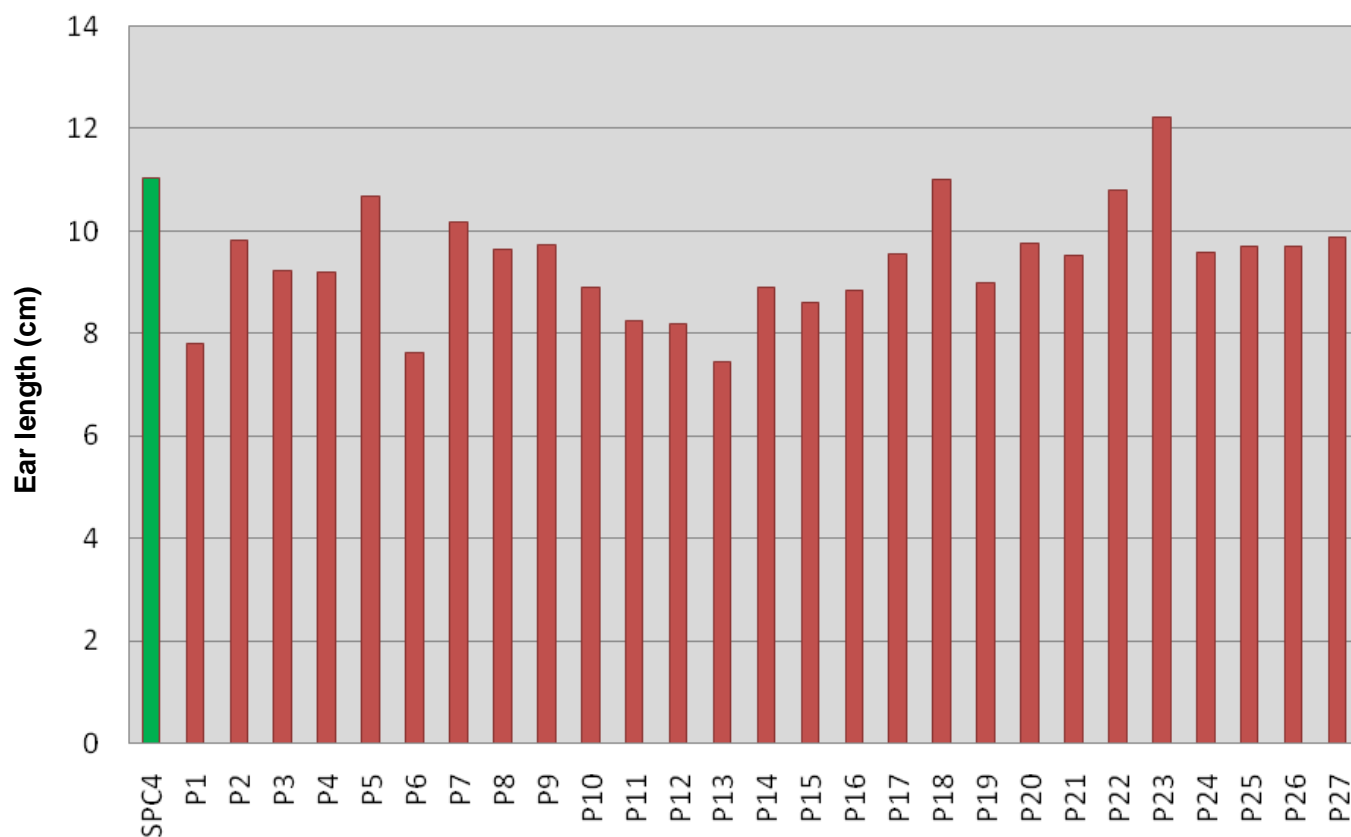
Figure 5. Distribution of the means of 27 synthetics from Iowa State University in comparison with SPC_4 for plant height at the haploid level (2009).

to separate the influence of allelic and non-allelic gene interactions on the manifestation of different traits in both theoretical and practical applications.

ACKNOWLEDGMENTS

Figure 6. Distribution of the means of 27 synthetics from Iowa State University in comparison with SPC$_4$ for ear length at the haploid level (2009).
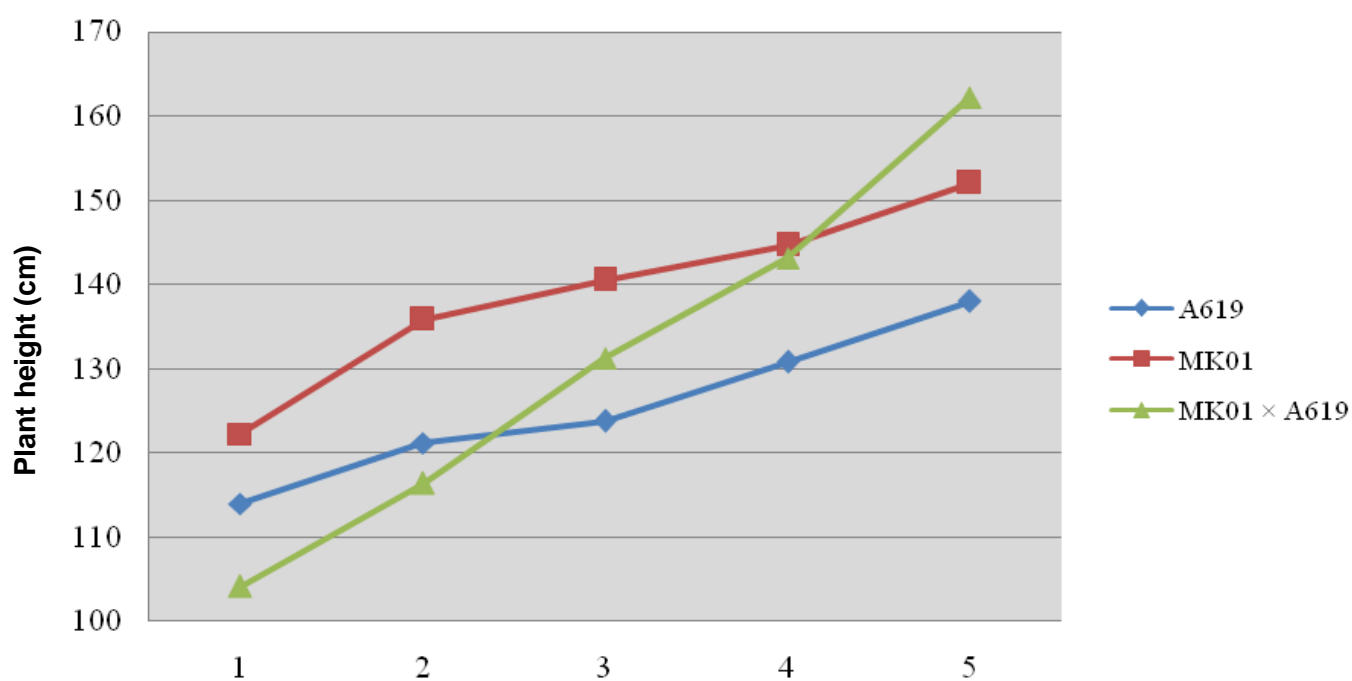

Figure 7. Distribution of the plant height means of five groups in the lines MK01 and A619 and their hybrid MK01× A619 at the haploid level (2009).
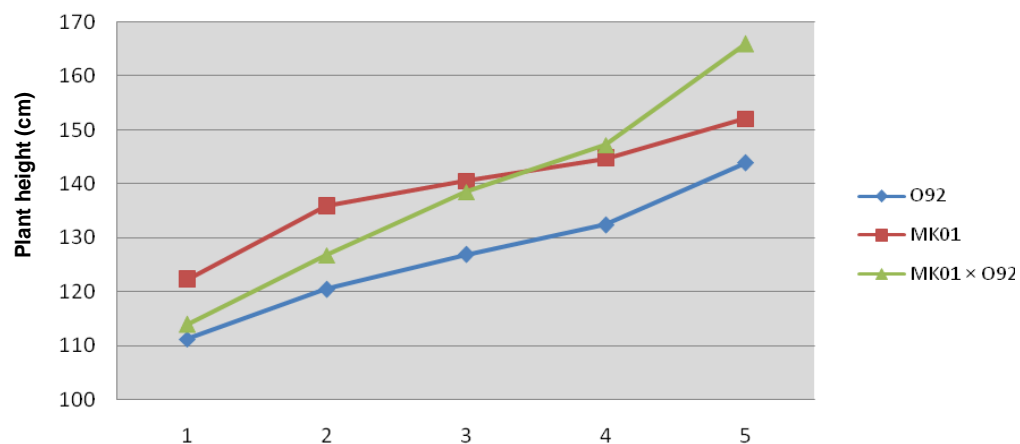
Figure 8. Distribution of the plant height means of five groups in the lines MK01 and 092 and their hybrid MK01× 092 at the haploid level (2009).
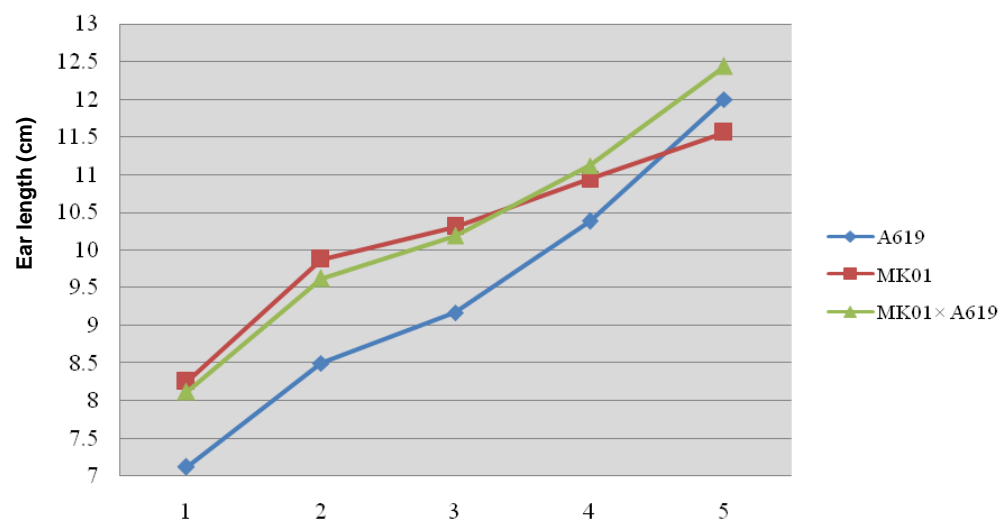

Figure 9. Distribution of the ear length means of five groups in the lines MK01 and A619 and their hybrid MK01× A619 at the haploid level (2009).
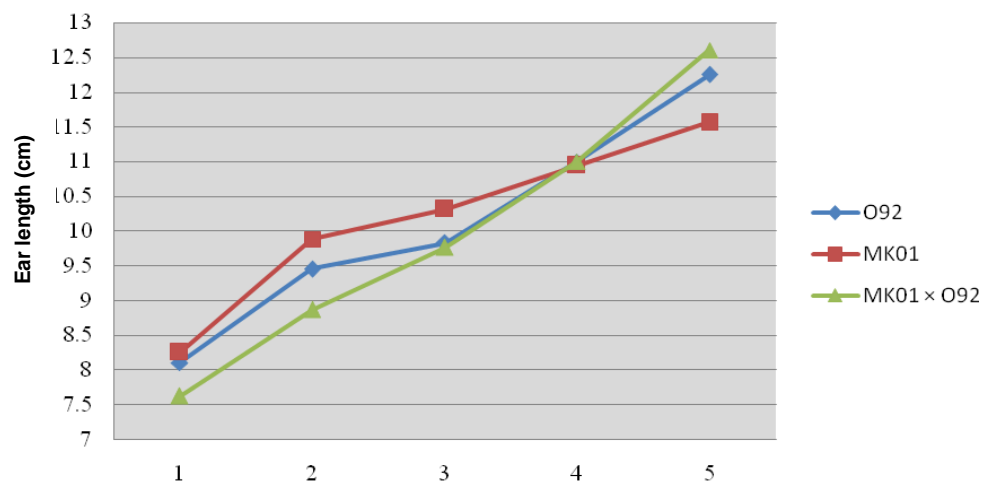

Figure 10. Distribution of the ear length means of five groups in the lines MK01 and 092 and their hybrid MK01 × 092 at the haploid level (2009).

REFERENCES

Chalyk ST (1994). Properties of maternal haploid maize plants and potential application to maize breeding. Euphytica, 79:13-18.

Chalyk ST (1999). Creating new haploid-inducing lines of maize. Maize Genet. Coop. Newslet., 73: 53-54.

Chalyk ST, Chebotar OD (1999). Utilizing maternal haploids to identify major genes controlling plant height in maize. Czech. J. Genet. Plant Breed., 35: 73-77.

Chalyk ST, Chebotar OD (2000). Regular segregation of recessive marker genes among maternal haploids in maize. Plant Breed., 119: 363-364.

Chalyk ST, Ostrovsky VV (1993). Comparison of haploid and diploid maize (*Zea mays* L.) plants with identical genotypes. J. Genet. Breed., 47: 77-80.

Chalyk ST, Rotarenco VA (1999). Using maternal haploid plants in recurrent selection in maize. Maize Genet. Coop. Newslet. 73: 56-57.

Chalyk ST, Rotarenco VA (2001). The use of matroclinous maize haploid for recurrent selection. Russ. J. Genet., 37: 1382-1387.

Chase SS (1952). Monoploids in maize. Iowa State College Press. Ames. IA. USA. pp. 389-399.

Chase SS (1964). Monoploids and diploids of maize: a comparison of genotypic equivalents. Amer. J. Bot., 51: 928-933.

Chebotar OD, Chalyk ST (1996). The use of maternal haploids for genetic analysis of the number of kernel rows per ear in maize. Hereditas, 124: 173-178.

Coe EH (1959). A line of maize with high haploid frequency. Am. Nat., 93: 381-382.

Geiger HH, Braun MD, Gordillo GA, Koch S, Jesse J, Krützfeldt BAE (2006). Variation for female fertility among haploid maize lines. Maize Genet Coop. Newslet., 80**:** 28-29.

Geiger HH, Gordillo GA (2009). Doubled haploids in hybrid maize breeding. Maydica, 54: 485-499.

Griffing BI (1975). Efficiancy changes due to use of doubled haploids in recurrent selection methods. Theor. Appl. Genet., 46: 367-386.

Hallauer AR, Miranda FJB (1988). Quantitative genetics in maize breeding. Iowa State University Press (Ames): 468.

Lashermes P, Beckert M (1988). A genetic control of maternal haploidy in maize (*Zea mays* L.) and selection of haploid inducing lines. Theor. Appl. Genet. 76: 405-410.

Lashermes P, Gaillard A, Beckert M (1988). Gynogenetic haploid plants analysis for agronomic and enzymatic markers in maize (*Zea mays* L.). Theor. Appl. Genet., 76**:** 570-572.

Nanda DK, Chase SS (1966). An embryo marker for detecting monoploids of maize (*Zea mays* L.). Crop Sci., 6: 213-215.

Röber FK, Gordillo GA, Geiger HH (2005). *In vivo* haploid induction in maize - Performance of new inducers and significance of doubled haploid lines in hybrid breeding. Maydica, 50**:** 275-283.

Rotarenco VA, Chalyk ST, Eder J (2004). Utilization of maize haploids plants in a recurrent selection procedure. J. Genet. Breed., 58: 61-72.

Rotarenco VA, Dicu G, State D, Fuia S (2010). New inducers of maternal haploids in maize. Maize Genet. Coop. Newslet., 84**:** 21-23.

Sarkar KR, Pandey A, Gayen P, Mandan JK, Kumar R, Sachan JKS (1994). Stabilization of high haploid inducer lines. Maize Genet. Coop. Newslet., 68: 64-65.

Shatskaya OA, Zabirova ER, Shcherbak VS, Chumak MV (1994). Mass induction of maternal haploids in corn. Maize Genet. Coop. Newslet., 68**:** 51.

Sprague GF, Eberhart SA (1977). Corn breeding. Corn and corn improvement. Agron. Monogr. 18. ASA, CSSA, and Madison. WI: 305-365.

Tyrnov VS, Zavalishina AN (1984). Inducing high frequency of matroclinal haploids in maize. Dokl. Akad. Nauk., 276: 735-738.