PHENOTYPIC ARIATIONS OF MAIZE CMS POPULATIONS AND SUBPOPULATIONS

2- YIELD TRAITS

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ABSTRACT

A study was accomplished to evaluate the contributions of selection and selfing in changing gene frequencies and genetic distinctness. Eighteen cms maize (*Zea mays* L.) populations were grown for six seasons during 2008-2010 at the field of Crop Science Dept./College of Agriculture. After three cycles of selection and selfing subpopulations retained significant reduction in means for most of the studied traits. Populations showed highly significant differences for all studied traits. The kernel weight reached its maximal values of 28.05g and 26.16g in A5o and R5o populations, and the hybrid A5sxR1o showed its maximal value in hybrids (32.03g). Genetic variability among parent populations concerning kernel weight results in different levels of hybrid vigor with hybrid phenotypes, which were of 83.38% greater than the best parent for the hybrid A5sxR6o. The highest yielding parent populations were of 62.35g and 101.3g for A1o from lines and R2o from tester populations, respectively. The hybrid combination A6sxR3o gave the highest mean for plant yield (141.5g). Selection was efficient in increasing hybrid vigor showed by some parental combinations, while it was acted differently as it reduced the ability of some populations to combine positively. However, hybrid vigor effects regarding the best parent were significant and A3sxR6s possessed its maximal value for plant yield which was 190.98%. Selection and selfing resulted in detectable alterations regarding the performance of populations per se and their ability to combine during hybridization process. These results were supportive to derive version lines with improved attitude.

المستخلص

لغرض تقييم مساهمة الانتخاب والتلقيح الذاتي في تغيير التكرار الجيني والتمايز الوراثي، تم إنماء ثمانية عشر من مجتمعات العقم الذكري السايتوبلازمي للذرة الصفراء على مدى ست مواسم في حقل تجارب قسم علوم المحاصيل الحقلية / كلية الزراعة. أظهرت المجتمعات المشتقة بعد ثالث دورات انتخابية انخفاضاً معنويا،ً كما أظهرت متوسطات الصفات المدروسة فروقاً عالية المعنوية. بلغ أعلى متوسط لصفة وزن الحبة في المجتمعات الأبوية (28.05غم) و 26.16غم) في A50 و R50، فيما اظهر الهجين A3oxR3s أعلى متوسط للصفة المذكورة في مجتمعات الهجن (17.87غم). أدى التغاير الوراثي بين المجتمعات الأبوية في صفة وزن الحبة إلى الحصول على قوة هجين بمستويات مختلفة في الهجن الناتجة من التضريب بين تلك اآلباء. إذ اظهر الهجين o6sxR5A أعلى قوة هجين للصفة المذكورة بلغ .%83.38تميزت الساللة o1A لدى إعطائها أعلى متوسط لصفة حاصل الحبوب للنبات بلغ)62.35غم(، فيما كان أداء اللواقح أفضل واظهر o2R أعلى متوسط للصفة ذاتها بلغ (101.3غم). أعطى الهجين A6sxR30 أعلى متوسط لصفة حاصل النبات (141.5 غم/نبات). كان الانتخاب فعالاً في زيادة قوة الهجين في بعض الهجن، فيما كان أداؤه مغايراً عندما قلل من قدرة بعض المجتمعات على إنتاج هجن ذات قوة هجين موجبة. على أية حال، كانت قيم قوة الهجين قياساً بالأب الأعلى معنوية، إذ حاز الهجين A3sxR6s على أعلى تلك القيم (190.98%) لصفة حاصل النبات. أدى الانتخاب بالتلقيح الذاتي إلى تبدل واضح في أداء مجتمعات الذرة الصفراء ومقدرتها على االتحاد خالل عملية التهجين. دعمت هذه النتائج إمكانية اشتقاق سالالت جديدة ذات أداء محسن.

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^{*} Part of ph.D Dissertation of the first

Introduction

Important increases in maize productivity have been obtained since the beginning of the last century because of the development of inbreeding and hybridization methods outlined by Shull [4]. Currently, most maize breeding programs are based on hybrid production. The development of inbred lines and hybrids is very much related to the frequency of favorable alleles, which can be increased via various selection methods [11], especially those dealing with developing populations and inbred lines to be crossed and form superior (elite) hybrids [16, 17].

The effective selection methods can cause changes in the allele frequencies, levels and distribution of the genetic variability, and consequently, the genetic structure of the populations [19]. The two keys to the successful breeding are variations and selection. In other words, all that any breeder really needs is some degree of genetic variation between the individuals in a given population, plus a means of identifying and selecting the most suitable variants. These more useful variants are then mated with each other to produce a population that is now composed almost entirely of the newly selected genetic population [15].

The laborious detasseling process can be avoided by using cytoplasmic male-sterile (cms) inbreds. Plants of a cms inbred are male sterile as a result of factors resulting from the cytoplasm. Thus, this characteristic is inherited exclusively through the female parent in maize plants, since most of the zygote cytoplasm provides by female parent [8]. This maternally inherited failure of a plant to produce functional pollen results from the expression of novel genes within the mitochondria [10]. Besides pollen sterility, cms can positively affect the yield potential [12], and result in a new genetic diversity eligible for elite hybrid development.

Studies at the molecular level revealed that cms genes are "chimeric" composed of short segments derived from various mitochondrial regions spliced together to give rise to new protein-coding genes. There are nuclear genes capable of suppressing CMS, each unique to a specific type of CMS [18]. In maize, these nuclear genes are designated as restorer of fertility (Rf) genes, which produce three major types of cms; T-cytoplasm (Texas), Scytoplasm (USDA), and C-cytoplasm (Charrua). T-cytoplasm type was the most extensively used because of the ease of finding suitable restorer genotypes and because of the complete and stable absence of pollen due to its asporophytic type [7]. The use of biotechnology in studying quantitative genetics on the molecular level has made significant contributions to develop more effective and efficient plant breeding systems for nearly all crop species [2].

Alvi et al. [1] investigated the performance of eight F1 hybrids of maize and cleared that the F1 hybrids exceeded their parents. The obtained values of hybrid vigor ranged from 21.44% to 34.41% and 8.81% to 33.04% for ear length and kernel weight, respectively. The largest amounts of hybrid vigor for yield component traits estimated by EL-Diasty [5], on the base of better parent were 72.33%, 41.30%, 19.70%, 16.46%, and 16.51% for ear weight, ear length, ear diameter, rows no/ear, and kernel weight, respectively.

By using procedures of classical genetic and quantitative trait loci (QTL) analyses, research was conducted by Frascaroli et al. [9] to study hybrid vigor, which was less than 50% for SE, PS, and kernel weight, 50% for plant height, 160% for SW and number of kernel per row, and even more than 200% for grain yield. The average level of better-parent hybrid vigor varied widely for the different traits (plant height, leaf angle, leaf width, stem width, cob diameter, cob weight kernel weight, and plant yield). The majority of traits exhibited hybrid vigor of 10%–30% [10]. Their results showed that plant grain yield and total kernel weight had the highest levels of hybrid vigor with

hybrid phenotypes more than 100% greater than the better-parent in both populations. It has been suggested that plant grain yield is a multiplicative trait that integrates variation from several other traits and therefore it may be expected that this trait would exhibit higher levels of hybrid vigor.

Materials and Methods:

Field experiments: Eighteen maize inbred lines were used in this experiment, and classified into three groups six lines of each, depending on its involving cms and rf genes: A-lines named A1 to A6, B-lines named B1 to B6, and R-lines named R1 to R6, all were in the fifth generation (S5). These genetic materials were of a long time work of Pro.M.M. Elsahookie at the same Dept. Seeds were grown at the field of the Dept. of Field Crop Sci./ College of Agriculture/ University of Baghdad, through six growing seasons of the years 2008 to 2010.

Field was prepared and recommended dosage of super phosphate fertilizer was incorporated in the soil after tillage operation at a rate of 400 kg P2O5 ha⁻¹. The recommended dosage of nitrogen fertilizer in the form of urea (46% N) was applied uniformly at the rate of 400 kg N ha⁻¹ in two splits each of 200 kg N ha⁻¹, the first was prior planting, and the second was applied when plants reach of height of 40-50 cm. Field was irrigated after planting as needed. The experiments were conducted under irrigated conditions and were kept free of weeds using herbicides (Atrazine, 4.5 litres ha⁻¹) and hand weeding. Corn borer was adequately controlled in all seasons at the stage of 6 leaves by using granular diazenon (10%) at the rate of 4 kg/ha.

Spring season/ 2008: Selection was initiated during the spring season of 2008. At least 100 seed of A-, B-, and R-lines were sown on 20 March 2008 at a wide space of two rows of each, 15 m long of 0.8 m between rows and 0.3 m between hills.

Ears were covered with transparent bags before the silks emerged to avoid open

pollination and were checked daily thereafter for silk emergence. Two days before pollination as silks reach the suitable length (5-6 cm), they have been cut back of the tip to guarantee full seed set formation. One day before pollination paper bags were used to cover the ready tassels which started shedding pollens. The pollinated ears were then recovered with a paper bag until harvest.

On the basis of some desirable traits (flowering time, tassel length, tassel branch number, stalk width, plant height, ear height, leaf area, leaf number, number of ears, ear length (as indicator for seed number per row), ear width (as indicator for row number), seed weight, and plant yield), selection was conducted on single plants of R-lines, and Blines, at the same time selected B-lines were crossed with A-lines to accomplish the first cycle of selection (S6). In order to maintain Alines, they were crossed with the corresponding maintainers. Original populations were propagated by sibbing.

Fall season/ 2008: The S6 seeds were planted on 27 August 2008. Field and growing plants were served as described before. Selection and selfing were performed by using the same procedure which has been followed in the last spring season. Selected single plants produced S7 seeds.

Spring season/ 2009: In order to produce S8 seeds, S7 seeds were planted in 27 March 2009. Last cycle of selection was applied and plants were handled in the same way as described in the previous cycle.

Fall season/ 2009: S8 seeds were planted, and sibbing among each population individuals was conducted to produce and stabilize the genetic structure of the new populations (subpopulations) of each entry in the experiment.

Spring season/ 2010: Both original and subpopulations were planted to conduct a series of crosses in all possibilities. To outcome of 144 F1's single cross, the original R-lines were crossed to both original and

subpopulations A-lines, while subpopulations R-lines have been crossed to both original and new A-line populations. A- and R-lines were sown in two dates (7 days interval) after the main sowing date to guarantee availability of fresh pollens adequate to do all possible combinations.

Fall season/ 2010: The 144 crosses and 24 parents were evaluated in the same trial during this season. All plots were hand-planted on 10 August 2010. Each plot consisted of three experimental units each of two rows of 4 m long and spaced 0.75 m apart, with hills spaced 0.19 m apart. Hills were overplanted then thinned at the 5- to 7-leaf stage to 1 seedling per hill, obtaining a final density of approximately $70,000$ plants ha^{-1} . Experimental units were distributed in a randomized complete block design with four replicates. Ten guarded plants from the middle of each row were sampled and data was collected for each entry for the following trait: Tassel branch number, kernels/row, rows/ear, kernel weight, and plant yield.

At physiological maturity (black layer formation) plots were manually harvested, then samples were dried and grain yield was adjusted to 15.5% grain moisture, and its components per plant were investigated too [3].

Plant leaf area was determined [6].

Statistical analysis:

Analysis of variance was carried out based on the data of individual plant to disclose the differences among populations, and the least significant difference test was then carried out on the level of (5%) to compare the means of the traits investigated.

Hybrid vigour of crosses was estimated as the percentage increase or decrease of F1 over the best parent [13].

Heterobeltiosis (H%)=[(F1 - HP)/HP] 100. Where F1 = performance of hybrid; HP = performance of best parent. Results and Discussion:

Yield components and plant yield: The longterm success of maize production was based on a constant increase in the average of yield components, which in turn increased average yield. Results indicated that means of all the entries were highly significant for all yield parameter. Populations were significantly different in ear number (Table 1), which its maximal mean reached 1.35 and 1.05 for A2s and R1o respectively, while its minimal mean reached 1 for six of A and the rest of R populations. Due to obvious genetic diversity among parent populations, hybrids showed different advantage over their parents. This advantage reached 1.4 for A2sxR1s hybrid. Estimates of heterotic effect indicated that the genes with over-dominance action of A6s and R3o parents were controlled the trait in their hybrid A6sxR3o as it possessed the highest level of hybrid vigor of 15% (Table 2). On the other side, partial-dominance action of A2s parents were controlled the trait in seven of their hybrids, which showed the lowest BP value of -25.93%.

Kernel rows number per ear (KR) reached its highest values of 14.6 and 17.3 in A3o and R6o populations respectively; meanwhile A6o and R1s possessed the lowest values of 12.2 and 12.1, respectively. These values indicated the magnitude of genetic diversity within and among parent populations, which in turn affected the strength of hybrids. Means tended to record higher values regard their parents and A1oxR6s was owned the highest mean of 18.7, whereas A4oxR1s hybrid owned the lowest one of 11.9. Type of gene action controlled this trait ranged from overdominance action in A3sxR6s hybrid which expressed the highest positive estimate of BP hybrid vigor (Table 2) of 34.6% and partialdominance in A5sxR3s hybrid, which expressed the lowest negative estimate of - 16.67%. Theses results agreed with those obtained by EL-Diasty [5].

Kernel number per row (KN) varied in the same manner as A4s and R5o populations

possessed the highest means of 25.95 and 27.7, respectively, whereas the lowest mean belonged to A3s and R1s populations of 16.75 and 16.45, respectively. As expected, hybrids differed significantly and obtained higher estimates for the trait in respect with their parents. These estimates ranged from 36.65 to 20.7 for A1oxR6s and A4oxR1s, respectively. Levels of BP hybrid vigor (Table 2) varied widely from an average of 68% for A1sxR1s to -14.68% for A3sxR3s. Frascaroli et al. [9] revealed introduced opinion. Parent populations expressed highly significant differences concerning kernel weight (KW). The trait reached its maximal values of 28.05g and 26.16g in A5o and R5o populations, while its minimal values were of 15.23g and 15.15g in A5s and R6o populations. Hybrids acted similarly as they differed significantly, meanwhile, they revealed higher means for KW trait ranged from 17.87g to 32.03g for A3oxR3s and A5sxR1o hybrids, respectively. Genetic variability among parent populations concerning kernel weight results in different levels of hybrid vigor with hybrid phenotypes, which were of 83.38% greater than the best parent for the hybrid A5sxR6o and of -22.77% lower than best parent for the hybrid A3sxR4o. These conclusions agreed with those obtained by Alvi et al. [1]; EL-Diasty [5] and Frascaroli et al. [9]. The highest yielding parent populations were of 62.35g and 101.3g for A1o from lines and R2o from tester populations respectively, while the lowest yielding populations were of 34.55g and 32.25g for A3s and R1s populations, respectively. Hybrids exceed the parent's average for plant yield and showed more diversity, which ranged from 141.5g to 49.2g for A6sxR3o and A3oxR3s hybrids, respectively.

Although subpopulations descended from the original ones, it was expected that their genetic

constitution will be identical and consequently their behavior with respect hybrid vigor. As a matter of fact, there is a significant alteration in the performance of population due to selection. Therefore, regarding the hybrid vigor with the best parent (Table 2), a wide range was noticed from 190.98% to -27.17% for A3sxR6s and A3oxR3s hybrids respectively, thus confirming the prevalence of alleles with increasing effects provided by R6s. Results indicated the effective selection of the additive genetic effects, which reduced the load of deleterious genes, increased the homozygosity of populations and produced less heterozygous hybrids that were characterized by improved yield potential per plant. These findings agreed with those reported by Frascaroli et al. [9] and Lippman and Zamir [14].

Molecular analysis of DNA revealed that the heterozygosity of the parents DNA could restrict the amount of variation that will affect the performance of their hybrids. Therefore, selection can create more distinctness among populations which is necessary in producing super hybrids. In the other hand, by crossing genetically divergent parents, the range of phenotypic variation will be much more extensive and can even be surprising as many hybrids like A3sxR6s and A3oxR3s for example, presenting phenotypes that would not be expected based on the attributes of their parents.

After three cycles of selection and selfing, it can be concluded that there is a detectable alteration concerning populations performance per se as well as their ability to combine and form hybrids. Therefore, it is so important to adopt effective selection programs in producing new version lines with improved performance.

Table 1: Mean estimates for populations and their crosses for studied traits in maize cms.

*EN=Ear number; KR=Kernel rows; KN=Kernel; KW=Kernel weight(g); PY= Plant yield (g).

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