A PROMISING APPROACH TO DEVELOP MAIZE IN BREDS AND TEST THEIR HYBRID PERFORMANCE

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ABSTRACT

A project to develop new maize (*Zea mays* L.) inbreds from inbred populations, crossed, and tested for performance. The project elapsed six seasons, 3 spring and 3 full plantings during 2015-2017. Newly developed inbreds showed significant grain yield increase as compared to the cross of its progenitor inbred. An increase of 2 t ha^{-1} was obtained with one of the new inbreds. The crosses obtained were evaluated in field trials with a registered hybrid (60×21). Method of selection counts on a unique plant trait in the inbred population, such as longer ear, thicker ear diameter, stay-green leaves, large kernel, and so on. The merit obtained due to hybrid vigor could be attributed to parental inbreds that have high number of SSR, genetically diverse loci or DNA methylation. The check hybrid (60×21) produced an average of 9.0 t ha^{-1}. Meanwhile, the cross 60×73 produced 9.01 t ha^{-1}, while the two newly derived inbreds produced higher grain yields, 60×73fr and 60×73dr which exceeded 11.0 t ha^{-1}. We have represented the case of hybrid vigor in a mathematical form; +1<1×1< -1. The next step of this program is to develop more inbreds from other inbreds and test their performance in field trials. At the same time, focus on the four crosses that performed more than 10 t ha^{-1}, these are namely; 60×73dr, 60×73fr, 74×844, and 74sg×73dw.

Keywords: hybrid vigor, selection, SSR, methylation, grain yield.

مجلة العلوم الزراعية العراقية -2018: 49(5):748-754 الساهوكي وآخرون

طريقة واعدة لاستنباط سلالات ذرة صفراء واختبار هجنها

مدحت مجيد الساهوكي مصطفى جمال الخفاجي عبد الباسط عبد الرزاق داود
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المستخلص

تم وضع برنامج لمشروع بهدف استنباط سلالات جديدة من الذرة الصفراء من مجتمع سلالات متوفرة واختبار أداء تضريباتها. استغرق عمل البرنامج ستة مواسم ، ثلاثة ربيعية وثلاثة خريفية خلال 2015 – 2017 . كان العمل في المواسم الربيعية للانتخاب والتلقيح الذاتي واجراء التضريب بين السلالات، فيما كان العمل في المواسم الخريفية اختبار اداء تلك التضريبات . طبق العمل في حقل قسم المحاصيل الحقلية السابق التابع لكلية الزراعة – جامعة بغداد . تم الحصول على معدل 2 طن هـ$^{-1}$ زيادة في حاصل الحبوب من احد تضريبات السلالة المشتقة من السلالة Zm73 . قورنت التضريبات بحسب تصميم القطاعات الكاملة المعشاة وباربع مكررات . كان الانتخاب لنباتات السلالات يتم استناداً الى صفات تظهر في نباتات السلالة مثل نبات طويل العرنوص او متأخر النضج او باوراق قائمة او كون الحبة كبيرة او منغوزة او صيوانية .... الخ . ان قوة الهجين التي تظهر في نباتات هجن الجيل الاول قد تعتمد على عدد SSR فيها او عدد المواقع الجينية المتغايرة وراثياً، كما ينعكس ذلك على سرعة معدل نمو النبات، والتبكير بالنضج واطالة المدة من الاخصاب حتى النضج، مع التبكير في النضج عن الابوين. تم تسجيل واعتماد التضريب 60×21 الذي انتج معدل 9 طن هـ$^{-1}$ تحت الري بماء بئر توصيله الكهربائي 2.5 ديسيسمنز وتربة فيها pH بحدود 7.5، فيما اعطى عند المزارعين معدل 13 طن هـ$^{-1}$ لما زرع في تربة جيدة وروي بماء عذب . قد تعمل DNA methylation في قوة الهجين بفعل epigenetic بوجود ترانسبوزون. تم تمثيل قوة الهجين بصيغة رياضية 1- >1×1<1+. كانت اربعة من التضريبات الجديدة متفوقة في الحاصل هي 60×73dr و 60×73fr و 74×844 و 74sg×73dw والتي اعطت معدل حاصل حبوب 11.15 و 11.10 و 10.30 و 10.25 طن هـ$^{-1}$ ، بالتتابع . استنادا لذلك نوصي باستمرار وتوسيع هذا البرنامج واختبار التضريبات الجديدة في مواقع اخرى ترصينا للنتائج.

الكلمات المفتاحية: الغزارة الهجينية، الانتخاب، SSR، حاصل الحبوب.

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INTRODUCTION

Inbreeding has been found to lower plant vigor traits and grain yield of plants. This could be attributed to different reasons. Ho et al (16) reported that selfing maize plants have reduced number of simple sequence repeats (SSR), and that was for an unknown reason. On the other hand, Liu et al (20) found that genetic distance and number of SSR were positively correlated in maize, and they have concluded that hybrid vigor of a maize hybrid counts on number of genetically diverged SSR among crossed inbreds. Hybrid vigor has been extensively used in the world agriculture for their better performance and biomass as compared to their parental inbreds. Lipman and Zamir (19) reported that a cross between two distinct species of tomato have produced an increased biomass. In general, genetic diversity between two species are more likely to be larger than between two inbreds of same species. Coverage of negative effects of some deleterious genes could be one of the reasons of vigor in the hybrids (28). There were different theories to explain hybrid vigour. Birchler et al (6) explained three mechanisms of gene action as reasons for hybrid vigour; dominance, overdominance, and pseudo-overdominance. Elsahookie (12) mentioned that dominance, semiepistasis coepistasis, and additive gene actions could be involved in hybrid vigour. Meanwhile, Singh et al (25) stated that genetic diversity among maize hybrids in corn belt of the US is still narrow for using genetic sources derived from 7 inbreds only; B73, LH82, LH123, PH207, PH595, PHG39, and Mo17. Traits of inbred plants contribute in some cases in vigour of their crosses. Weight and shape of F_1 seeds are mostly controlled by female parent, except in case when female parent is saccharta (11 , 29). Elsahookie (13) found that growth rate of hybrid maize was positively correlated with grain yield. Liu et al (21) found that 5 genes were controlling kernel traits in maize, meanwhile, Tao et al (26) identified 97 heterotic loci in a hybrid, and that was 33 loci governed four traits in rice plants (17). Abd and Elsahookie (1) found that leaf chlorophyll content at maturity of hybrids was higher than in their parental inbreds, and that was correlated to higher growth rate and grain yield in the hybrids (2 , 18). On the molecular level, Greaves et al (14) revealed in their work, that cytosine methylation hybrids of Arabidopsis were different than their parental lines. Groszmann et al (15) reported that hybrids had changes in defense and stress response gene expression that could be contribute to greater growth in the hybrids. The objectives of this study were, to develop new inbreds from inbred populations counting on some unique agronomic traits appear on some inbred population plants such as late flowering, heavier kernel, longer ears …etc.(4 , 5). These plants were selfed , seeds increased then crossed to an inbred, and then, their crosses evaluated in field trials with one or two checks.

MATERIALS AND METHODS

At the ex-farm of the Field Crops Dept., College of Agric. Univ. of Baghdad, a piece of land was prepared and divided as furrows in spring, and plots of 3×4m in the fall. In spring, seeds of available inbreds were planted in hundreds for each inbred population, then unique plants were identified, and selfed (3). At the same time, when new inbreds seeds became enough, crossing was done in next season. The program started in spring 2015 and ended in fall 2017, involved three springs and three full plantings. The inbreds were started on, Zm4, Zm17, Zm19, Zm21, Zm51, Zm60, Zm61, Zm73, and Zm74. These inbreds were developed years ago through several generations of selection, at least, not less than eight generations for each inbred. Crossing was conducted according to previous information on inbreds. There were 10 crosses evaluated in fall 2015 plus two checks; a synthetic 5018, and a Spanish hybrid. Planting was in mid March and mid July for spring and fall, respectively. The plots (4×3m) consisted of 8 rows for two crosses, 4 rows for each cross. This was in a randomized complete block design of 4 replicates. Spacing were 50×25 cm giving a population density of 80,000 plants ha^{-1}. There were 14 crosses under evaluation in 2016, and 23 crosses in fall 2017. When plants about 15 cm high, malathion was sprayed as recommended in the label. Fertilizers (as available) was applied twice, first when plants about 20-25 cm using urea (46% N) in a rate of 200 kg N ha^{-1} top dressed. The second time of fertilization was when plants reached 40 – 50 cm in height. Grooves were done 10 cm aside the plants, and 200 kg ha^{-1} of compound fertilizer (18 – 18 – 18) was side-dressed, then covered with soil. Each season, the herbicide guardian was used after planting as recommended. Soil pH was around 7.5, and irrigation was practiced as needed. The source of irrigation water was from a well with 2.5 dS m^{-1}. Measurements on plants in the fall seasons were done on 5 plants of each experimental unit. Leaf area was estimated by measuring the length of leaf below ear leaf, squared, and multiplied by a factor (10). Chlorophyll indices of plant leaves were taken by using Spad refractometer. Other agronomic traits were also recorded as they appear in data Tables. At maturity, 5 marked plants were harvested, air dried, and threshed. Values of grain yield, and yield components were done for each experimental unit. Data tabulated and analyzed according to the design used. The means were compared using LSD.

RESULTS AND DISCUSSION

Results obtained on plants from crosses evaluated in fall 2015 are shows in Table 1. Correlation coefficient between grain yield and plant traits was not done, for that test requires enough data for each pair of traits to be tested. However, it was so difficult to name a single agronomic or phenotypic trait explains heterosis of a hybrid, since many traits complemented each other to drive the heterotic process are required (7,8,9,20,21,23,24,27). An elite hybrid should have at the same time at least some positive traits complementing each other, such as high growth rate, efficient leaf area, long ears, high nitrogen and chlorophyll content in the leaves at maturity. In such a case, we expect stay-green hybrids plants are candidate for higher performance. Almost always, elite hybrids should have high number of kernels in unit of area, and heavier kernel weight, or at least a moderate kernel weight.

Table 1. Traits of maize crosses tested in fall 2015

No.	Crosses	Plant height cm	Ear height cm	Leaf area m^2	Chloro. index Spad	Row/ ear	Ear length cm	Kernel/ ear	Kernel/ weight mg	Grain yield t ha^{-1}
1	Syn. 5018	170	78	0.51	51	16	18.0	496	218	7.15
2	Span. hybrid	155	65	0.48	48	14	18.0	446	262	7.61
3	60×21	175	70	0.51	52	16	19.0	544	245	8.73
4	17×60	154	68	0.43	48	18	17.1	594	220	8.30
5	73×21	160	65	0.47	49	16	17.0	640	198	8.24
6	73×4	162	66	0.53	46	16	17.5	560	240	8.45
7	73×17	175	70	0.43	54	18	16.8	558	232	8.54
8	73×19	158	60	0.46	48	14	16.5	476	245	7.52
9	73×51	162	61	0.51	51	16	19.3	576	227	8.52
10	73×60	164	62	0.50	49	16	19.0	544	256	9.01
11	73×61	163	65	0.50	47	16	17.4	480	236	7.15
12	73×21	162	66	0.51	45	12	19.5	432	225	6.31
	Lsd 0.05	006	05	0.05	04	01	1.5	044	022	0.63

As shows in Table 1, plant heights of crosses were around an average of about 165 cm, although there were significant differences. Similar differences are exist in ear height. Plants of higher ear position are more likely to be lodging susceptible, and have lesser leaves above ear. This means that the source will be less than similar leaf area of other hybrid plants of lower ear position. Chlorophyll index did not show a linear positive relationship with grain yield. Longer ears are preferred in the hybrids, but they should have high number of kernel rows, and kernel number. If we look for kernel number of ears and kernel weight (Table 1), there were four crosses, namely; numbers 10, 3, 7, and 9 of higher grain yields, and higher number of kernels and / or heavier kernel weight. Grains of parental inbreds of these four crosses were increases and evaluated later as compared with a check hybrids. Three of these crosses, including the cross (60×21) were sent to a committee in Ministry of Agriculture for registrations. Only the cross (60×21) was registered for release by that committee, and the Iraqi Seed Co. have handed the seeds of the two parental inbreds to Ministry of Agriculture for propagation, and production of F_1 seeds for commercial use for maize Iraqi growers.

Table 2. Traits of maize crosses tested in fall 2016

No.	Crosses	Plant height cm	Ear height cm	Leaf area m^2	Stalk cliam. cm	Leaf/ plant	Days to tassel	Days to silk	Days to mat.	Chloro. index Spad	Grain yield t ha^{-1}
1	4×60	145	61	0.50	1.25	14.5	59	64	28	49	7.68
2	60×4	141	60	0.48	1.40	14.5	60	64	30	47	7.61
3	21×74	155	65	0.49	1.40	15.0	59	65	30	44	7.38
4	60×21	140	58	0.48	1.25	16.0	58	64	29	49	8.10
5	60×51	149	63	0.48	1.30	15.0	60	63	31	47	8.37
6	51×60	151	65	0.46	1.40	15.0	58	64	28	45	8.07
7	17×4	160	66	0.46	1.30	16.0	59	64	33	50	7.74
8	51×17	149	64	0.42	1.30	16.5	59	64	29	49	7.68
9	21×51	145	58	0.44	1.40	15.5	59	64	31	49	6.95
10	4×51	152	55	0.49	1.35	16.5	60	65	31	52	7.63
11	4×21	151	52	0.52	1.50	15.5	58	62	31	41	8.35
12	17×60	148	52	0.42	1.25	14.5	60	64	30	47	7.51
13	4×74	155	60	0.45	1.35	16.0	63	67	30	50	6.53
14	51×74	149	58	0.47	1.41	14.5	63	66	29	51	7.02
	Lsd 0.05	007	06	0.02	0.13	0.6	01	02	02	03	0.26

It is worthy to mention that grain yield of the new registered hybrid had about 9.0 ha^{-1} grain yield, but at the same time, when we got enough F_1 seeds, it produced 13 t ha^{-1} on farmers farms, due to better soil pH and irrigating with river fresh water. The phenotypic and agronomic traits of 14 maize crosses evaluated in fall 2016 are shows in Table 2. Plant heights of plants ranged between 140 to 160 cm with significant differences among them. The check hybrid (60×21) had the shortest height (140 cm). Seeds of F_1 crosses of better grain yields in Table 1 were not enough to grow in this season. However, other crosses not grown last year are shown (Table 2). Ear heights were corresponding to plant heights. They range from 52 cm of cross (17×60) to 66 cm of the cross (17×4). The cross (4×21) had the widest plant leaf area (0.52 m^2) with thickest stalk diameter (1.5 cm). The anthesis to silk interval (ASI) of this cross was 4 days (52 to 62 days to silk) and elapsed 31 days from full silking to physiologic maturity. This cross had a high value of grain yield (8.35 t ha^{-1}) and not significantly different with grain yield of the cross (60×51) which produced a similar value (8.37 t ha^{-1}). At the same time, these two crosses were significantly higher in grain yield than the check hybrid (60×21) which produced 8.16 t ha^{-1}. Values of chlorophyll index were not corresponding linearly with grain yield. This could be explained in part due to probable differences in leaf efficiency to produce net assimilation rate. High temperature, coincided with low humidity and dusty days dominated that season had negative effects on plant growth and performance. We did not have statistics for these parameters of weather in area, but at last 50% of the days of growth season were very hot (over 50 C) dry air and dusty. There were 23 crosses evaluated in fall 2017, this year had better weather than the year before (Table 3). The first cross in the Table is check hybrid (60×21), there are from last year two new inbreds derived from inbred 60; 60fw and 60fr. The crosses of these two inbreds are shown directly after the check. One of them gave similar grain yield to the check, while the second gave less. The two new inbreds (60fw and 60fr) were crossed to new inbreds derived from Zm73, they were 73dw and 73fw. These crosses are shown in numbers 5-8. There were significant differences in grain yields, but they were not the best in this trial. Another example about the newly developed inbreds, 17A which was selected from Zm17. Numbers 13 and 14 (Table 3), 17A×844 and 17×844 were significantly different in grain yield. 17A×844 produced 7.80 while 17×844 produced 10.03 t ha^{-1}. This confirms the negative response of the selected inbred (17A).

Table 3. Traits of maize crosses tested in fall 2017

No.	Crosses	Plant height cm	Ear height cm	Leaf area m^2	Chloro. index Spad	Row/ ear	Ear length cm	Kernel/ ear	Kernel/ weight mg	Grain yield t ha^{-1}
1	60×21	157	75	0.400	39	15.0	16.8	530	227	9.61
2	60fw×21	153	73	0.411	45	17.8	16.0	649	180	9.55
3	60fr×21	154	71	0.474	37	17.8	15.6	447	216	7.78
4	60×844	149	74	0.514	42	16.8	15.8	494	209	8.25
5	60fw×73dw	152	69	0.454	44	16.5	18.3	549	203	8.90
6	60fr×73dw	156	69	0.422	44	16.3	16.8	541	167	7.20
7	60×73fw	171	78	0.445	47	18.8	18.8	678	170	9.23
8	60×73dw	173	81	0.425	44	17.3	16.3	661	148	7.84
9	834×21	168	85	0.414	40	14.0	16.0	601	193	9.28
10	834×844	151	82	0.414	39	15.5	16.5	328	276	7.30
11	61×73dw	159	77	0.428	41	17.5	16.9	540	181	7.80
12	61×73dr	153	70	0.434	39	15.8	17.4	424	230	7.40
13	17A×844	135	67	0.486	36	14.5	16.0	343	284	7.80
14	17×844	153	88	0.496	42	15.8	15.3	513	245	10.03
15	17×73dw	154	85	0.442	49	17.8	13.1	482	170	6.55
16	74sg×21	164	91	0.477	38	17.8	16.4	552	207	9.15
17	74sg×73dw	181	99	0.578	33	18.0	17.8	575	223	10.25
18	74×21	174	98	0.480	35	15.5	17.5	497	230	9.20
19	60×73fr	163	83	0.439	45	18.3	16.6	713	195	11.10
20	60×73dr	190	72	0.403	47	17.5	15.9	672	212	11.15
21	17D×73fw	118	95	0.532	45	16.8	17.1	586	207	9.70
22	17E×844	146	63	0.420	30	13.8	13.6	335	277	7.40
23	74×884	146	76	0.373	32	17.0	17.4	606	213	10.30
	Lsd 0.05	008	06	0.046	03	1.8	1.0	107	27	1.70

This inbred had vigorous plant growth and produces very long ear with high kernel number, but unfortunately of no specific combining ability with inbred 844. Another example of newly selected inbreds is that of 74, which is coded 74sg (that is for stay-green leaves). When we compare their performance (numbers 16 and 18), it was found no significant difference (9.15 and 9.2 t ha^{-1}). At the same time, same new inbred (74sg) gave high grain yield better than many other crosses including the crosses (74×21 no. 18) and (74×21 no. 16). It has have seen from this overview, all derived inbreds from inbreds 17 and 60 were of low combining ability with inbreds crossed to, but we can not tell if they will not do better if crossed with some other inbreds. Meanwhile, the new inbred (74sg) did not produce well when crossed with inbred 21 (no. 16), it gave 9.15 t ha^{-1}, while it was produced 10.25 t ha^{-1} when crossed to a new inbred (73dw), crosses no. 16 and 17. There were so many probabilies of crossing. Meanwhile, about the cross (73×60) in Table 3 (no. 10), which produced 9.01 t ha^{-1}, it was the same with its reciprocal (60×73). Here we come to most important significant merit of our program approach, the inbreds derived from the inbred Zm73, they were 73fr (flint kernel with red cob), 73dr (dent kernel with red cob), 73dw (dent kernel with white cob), and 73fw (flint kernel with white cob). These four newly derived inbreds were crossed to inbred 60. Numbers 7 and 8 (60×73fw) and (60×73dw) produced 9.23 and 7.84 t ha^{-1}, respectively. They were significantly difference, but the other two crosses were the best, they are no. 19 and 20, crosses (60×73fr) and (60×73dr) which produced 11.10 and 11.15 t ha^{-1}, respectively. When we go back to the original cross (73×60) in Table 1 which produced 9.01 t ha^{-1}, it is notice the significant and remarkable difference between performance of these two new inbreds when crossed to the same inbred. If phenotypic characters, such as flint, or dent kernel, red or white cob, sessile or sharnked ears … are linked or correlated to some SSR or favorable loci, the selection will be beneficial. To be sure of that, we have to test all possible probabilities. However, the new discovery of four-helix DNA in human cell nuclei could open a new era to study the plant genome more precised (30). Number of complementing genetically diverged SSR in the hybrids lead to have higher performance (20). Ho et al (16) found that selfing is a major reason that inbreds loose some of SSR as compared to their open-pollinated progenitors. This implies that high number of SSR in crossed inbreds, and / or heterotic loci are important for an elite hybrid (24, 26). Scheuring et al (24) studied the famous US hybrid (B73 ×Mo17) and its inbreds and found that 800 genes in the hybrid were increased in their expression about two to ten folds as compared to its parental inbreds. Efficient leaf area, high nitrogen content in the leaves at maturity, high growth rate, longer ear in general would lead to have high kernel number in unit of area and heavier or moderate kernel weight then a high performance hybrid will be expected. This project showed encouraging results to reselect new inbreds from some promising inbred populations counting on some phenotypic and / or agronomic traits found on some unique plants in those populations. Detailed data on these inbreds needed so well, but to do that we have to have better budget for better job. We conclude that this program should be expanded to cover more inbreds under selection, more selected and selfed plants, and do all diallel crosses. More than one or two locations are needed for preliminary yield trials.

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