

Flowering Syndrome-Hybrid Performance Relationship in Maize 2- Grain Yield and Yield Components

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Abstract: A field experiment was conducted for 4 seasons on the farm of the Dept. of Field Crop Sci., Coll. of Agric., Univ. of Baghdad in spring and fall plantings in 2014 and 2015. That was to determine the relationship of hybrid performance in maize (*Zea mays L.*) crosses with early and late selects of inbreds. Four inbreds; Zm19, Zm32, Zm51, and Zm61 were grown and the very early and very late silking plants were selected and selfed for propagation in the first two seasons. The third season involved growing the selects and top-crossing with early and late inbreds (Zm60 and Zm21). The sixteen crosses were planted in season 4 in RCBD of 3 replicates in population density of 83'000 plants. ha⁻¹. The cross (Zm19xZm60) resulted from early select of Zm19 gave significantly higher grain yield (10.52 t. ha⁻¹) compared to its late counterpart (8.19 t. ha⁻¹). The same cross gave higher grain yield than late Zm19 crossed to late inbred (Zm21) (6.64 t. ha⁻¹). Early selects on inbreds crossed to testers showed significant differences in kernel growth rate (KGR), kernel filling duration (KFD) and kernel weight. Values of KGR ranged between 3.2 - 3.5 g. plant⁻¹. d⁻¹, KFD between 35 – 38 d, and kernel weight between 228 – 294 mg. kernel⁻¹. It was concluded that selection on maize inbred populations creates new variations in traits lead to higher grain yield hybrids. Other traits such as ear length, kernel. ear⁻¹, and kernel weight could be good candidates for selection on inbreds that could help developing new high grain yield hybrids.

Keywords: Days of Kernel Filling, Epigenetic, Grain Yield, Kernel Growth Rate, QTL, Selection on Inbreds

1. Introduction

The transition process from vegetative to reproductive stage in plants is of prime importance to productivity, since this process is influenced by genetic and environmental factors [1]. The time of flowering is a complex syndrome that shows a wide range (35 – 120 days) in maize [2]. Time of flowering in plants could be related to some phenotypic traits such as plant height, leaf area, and seed filling [3]. Several researchers [4, 5, 6] reported over 60 QTL in *maize* and *Arabidopsis*, and that each QTL in maize controls a part of phenotypic character [5]. In maize, there are genes control flowering, but still sharing control on other traits [5,7,8]. Durand et al. [9] worked on selection for flowering time in maize population and found 4 d interval between flowering time of two selected groups. On the other hand, Chardon et al. [10] reported some mutations in maize flowering time, which was correlated with meristem differentiation from vegetative to reproductive stages.

Hybrid vigour in maize and other crops is of wide international uses and highly important in world food production. However, this phenomenon is still not well understood. Meanwhile, hybrid vigour counts on wide genetic diversity between crossed parents [11]. Molecular discoveries obtained in the last years are considered as a beginning to understand this phenomenon [12, 13]. Allelic interactions of parents are well-thought-out responsible on growth rate, biotic and abiotic tolerance in hybrids [14]. The regulative actions of epigenetic effect are considered so important in understanding the actions and interactions between DNA and siRNA in hybrid vigour activities of growth rate and phenotypic traits [12, 15, 16, 17, 18]. The objective of this trial was to discover the relationship between flowering syndrome (early and late flowering) selection on some maize inbreds populations and the performance of hybrid crosses resulted from those two selects by using two diverged inbreds in flowering time.

2. Materials and Methods

A field experiment was applied on the farm of the Dept. of Filed Crops Sci, Coll. of Agric., Univ. of Baghdad in four seasons (spring and fall) in 2014 and 2015. Four maize inbreds (Zm19, Zm32, Zm51, and Zm61) were grown and early and late silking plants were selected and selfed. In the second season, the eight groups of seeds were grown for propagation via selfing. In the third season, the seeds of the eight selected groups were grown and top-crossed to early silking inbred (Zm60) and to late silking inbred (Zm21). The sixteen crosses seeds were grown in the fourth season in RCBD of three replicates. Each plot consisted of six rows (7.5 m²) of 83'000 plants. ha⁻¹. Fertilizers used were 200 kg/ha of each N, P and K. Fertilizers sources were urea, triple superphosphate, and potassium sulfate, respectively. Practices of weeding and irrigation were done as needed. Data were recorded on plants of the central four rows. Samples of 10 plants of each plot were taken to record data on agronomic traits as shown in the tables of this part and part 1 of the article published in this issue of this journal. Results obtained were tabulated and statistically analyzed.

2.1. Rapid PCR Test

RAPID polymerase Chain Reaction (PCR) test was conducted for some superior crosses in grain yield (Zm19xZm60 and Zm61xZm21 early and late flowering) to find out the nature of DNA bands of each group of the late and early flowering superior crosses, DNA was extracted from the seedlings of the above mentioned crosses and specific segments of DNA were amplified using 15 primers [19].

2.2. PCR Requirements

The main requirements of PCR are:

- Tag DNA Polymerase.
- DNA Primers, Deoxynucleosid triphosphates, which are nucleosides of (Deoxy Adenosine Triphosphate-dATP; Deoxy Thymosine Triphosphate-dTTP; Deoxy Guanosine Triphosphate-dGTP; and Deoxy Cytosine Triphosphate-dCTP).
- PCR buffer including Mg⁺⁺.
- DNA template, Thermocycler.
- Other components [20].

2.3. PCR Stages

Three basic stages were identified in PCR reaction, which are repeated in each cycle of DNA replication cycles over a limited period of time [20]. The stages are:

1. Denaturation of the two strands of DNA: by raising the temperature of the reaction solution to 92-95°C for 3 to 5 minutes, the double helix of DNA will be melted apart to create single strand that will be a template to build a complementary segment of it.
2. Primer annealing: in this stage the temperature will be lowered to allow the primers to bind the single strand of

DNA template. The best primer binding temperatures vary but it is typically around 60°C.

3. Extension; the last stage of PCR reaction, which includes adding dNTPs to the end of OH of the primer at the binding location with DNA template to produce new strand (a complementary DNA strand) to the sequence target. Best temperature required for this stage is 72°C.

3. Results and Discussion

Kernel weight and number. m⁻²:

Kernel weight is determined via size of source of metabolites and its efficiency to be converted into kernel components [21]. Kernels start developing after fertilization, continue in growth until dough stage and reach their final weight at time of physiologic maturity [22]. However, kernel weight is highly inherited in the genotype, and genotypes differ in this trait [23, 24, 25]. Those differences in final kernel weights of genotypes are related to system capacity constant in the genotype, which is associated with the traits contribute to dry matter accumulation [24, 26, 27]. Table 1 shows significant differences in kernel weights of crosses. The cross [19x60) showed significant difference between early and late silking select crosses.

Table 1. Kernel weight (mg) and kernels. m⁻² of crosses of early and late selects of maize inbreds obtained from crossing them with two testers (late Zm21 and early Zm60).

Crosses	Selected populations			
	Early	Late	Early	Late
	Kernel weight		Kernel. m ⁻²	
19x21	199	171	3328	3349
32x21	208	191	3697	3839
51x21	250	245	4037	4056
61x21	217	240	4561	4565
19x60	294	191	3591	4296
32x60	244	205	4232	4513
51x60	232	225	4317	4145
61x60	228	215	4519	4758
Lsd				
5%	040		847	
10%	030		704	

The cross (32x60) gave a significant difference in kernel weight at p=0.1. Other select crosses were similar in kernel weights for early and late. On the other hand, early select cross of inbred Zm19 was significantly different when crossed with late (Zm21) and early (Zm60) testers. Since the cross 19x21 gave 199 mg/kernel compared to 294 mg/kernel of the cross 19x60.

Number of kernels. m⁻² of crosses were also significant as shown in Table1. Crosses in general were significantly different in this trait. However, early and late selects from inbred Zm19 crossed to early inbred tester (Zm60) showed significant difference in number of kernels. m⁻². At the same time, these two selects did not give significant difference when crossed to late inbred tester (Zm21). The cross 19x60 gave 3591 and 4296 kernel. m⁻² for late and early selects,

respectively. As it will be shown later, this cross did not give higher grain yield. That could be due to low kernel growth rate and low number of days for kernel filling. Number of kernels. plant^{-1} or. m^{-2} is an important component in grain yield in all seed plants [24, 28, 29].

Days of kernel filling and kernel growth rate:

Days of kernel filling (DKF) and kernel growth rate (KGR) are the components that determine the final kernel weight [20]. Genetic and growth variables are controlling DKF and KGR. However, it could be possible to manage DKF by optimizing dates of planting, fertilization, and irrigation. Tollenaar *et al.* [31] reported that grain yield of maize could be increased by 0.37 t. ha^{-1} for each extra day of DKF. On the other hand, Greaves *et al.* [15] described that some maize early hybrids gave higher grain yield because of high KGR. Data of Table 2 shows that all early and late select crosses gave significant differences in DKF except the cross 51x21 that gave 38 d for both late and early select crosses.

Table 2. Kernel growth rate (KGR) $\text{g. plant}^{-1} \cdot \text{d}^{-1}$ and days of kernel filling (DKF) of maize crosses resulted from early and late selects of inbreds crossed by two testers (late Zm21 and early Zm60).

Crosses	Selected populations			
	Early	Late	Early	Late
	KGR		DKF	
19x21	2.1	1.9	38	35
32x21	2.3	2.4	40	36
51x21	3.2	3.1	38	38
61x21	3.0	3.7	40	35
19x60	3.4	2.9	37	34
32x60	3.3	3.2	38	35
51x60	3.1	3.1	38	36
61x60	3.3	3.5	37	34
lsd				
5%	0.7		2	
10%	0.6		2	

Early selects of inbreds when crossed to early or late inbred showed about 3-5 d longer time for DKF. If other related yield components are remained the same, grain yield will be significantly increased.

In general, we can note from Table 2 that crosses of longer DKF had lower KGR, but this is not a rule. The best two opposite crosses in this trend are 61x21 and 19x60, since they had 40, 35 DKF and 3.0, 3.7 KGR, for cross 61x21, and 37, 34 DKF and 3.4, 2.9 KGR for the cross 19x60. Values of KGR were not significantly different between crosses of early and late selects except for the cross 61x21 that late select cross had 3.7, and early select cross has $3.0 \text{ g. m}^{-2} \cdot \text{d}^{-1}$ KGR. Some researchers [15] reported that they discovered many QTLs in maize related to DKF and KGR, and ultimately they will play an important part on grain yield.

Grain yield:

Grain yield per plant or per unit area is the final output of any grown crop under defined growth variables. Grain yield is the outcome of the different actions and interactions of genetic and environmental parameters, which is represented by the kernels number and its weight [24, 32, 33]. Results of

grain yield in Table 3 show that there were significant differences among crosses in grain yield per m^2 and per ha.

Several researchers gave some explanations on maize hybrid grain yields that are influenced by epigenetic [34, 35, 36, 37, 38]. Ni *et al.* [37] reported that changes in gene expression were related with crop growth rate in *Arabidopsis thaliana*. This could lead to differences in seed yield of plant genotypes. There was an important remarkable note about early and late selects of inbred Zm19 when crossed to the late inbred tester (Zm21). This cross (19x21) gave lower grain yield of both early and late selects as compared to the same selects crossed with early inbred tester (Zm60). These two crossed (early and late 19x21) gave only 6.642 and 5.621 t. ha^{-1} , respectively. This could be due to a different combining ability with that inbred (Zm21). In general, different and genetically diverged inbreds give different combining abilities. It was noticed in data of Table 3 that early selects crossed to early tester (Zm60) had higher grain yield than when crossed with late tester (Zm21).

Table 3. Grain yield (kg. m^{-2} and t. ha^{-1}) of maize crosses of early and late inbred selects obtained from crossing them by two testers (late Zm21 and early Zm60).

Crosses	Selected populations			
	Early	Late	Early	Late
	Kg. m^{-2}		t. ha^{-1}	
19x21	0.66	0.56	6.642	5.621
32x21	0.77	0.74	7.691	7.370
51x21	1.02	1.00	10.214	9.952
61x21	0.99	1.09	9.901	10.899
19x60	1.05	0.82	10.516	8.188
32x60	1.03	0.92	10.307	9.158
51x60	1.00	0.94	10.029	9.386
61x60	1.02	1.00	10.199	10.024
lsd				
5%	0.21		2.065	
10%	0.17		1.716	

It could be due to the genes of early and late flowering and/or combining ability. Durand *et al.* [3] stated that flowering time is a complex trait and important in determining yield components, and ultimately grain yield. They reported that they found a series of alleles on one locus of flowering, and this will lead to variations in plant and kernel growth rates that could be related to grain yield.

The differences in growth and yield parameters among hybrids are not necessarily to be governed by DNA only. Epigenetics is pertained to the change in gene expression without a change in DNA nucleotide sequences. Some researchers [39, 40] mentioned that siRNAs of 21-24-nt play prime role in novel mutations, some of them are related to late flowering. Shen *et al.* [18] stated that DNA and siRNA are involved in hybrid vigour. Tollenaar [31] reviewed very good ideas on the role of epigenetics in hybrid performance. They stated that DNA methylation and histone-modification are negatively related with hybrid grain yield. Transposable elements and repeated elements play an important part in regulating gene action. This role will be through activation or silencing of some genes. As a result of that, imprinting will

take place, and then epimutation appears (in the hybrid) leading to significant and remarkable differences over parental origin.

The results of this article showed that selection for some important traits on inbreds such as flowering could lead to new variations in the selected populations. These variations lead to significant variation in growth parameters of plant and kernel. This will ultimately give significant higher hybrid grain yield. Other traits, such as; ear length, heavier kernel weight, higher ear growth rate, and others may be of highly importance variations in inbred populations that lead to better hybrid performance. Selection for those traits suggests growing large inbred populations (e.g 5000) plants for each inbred. This will increase the probability of variations for better selections.

Molecular genetics results – PCR:

PCR test results shown DNA bands (using primer OPE-13) of the cross Zm19xZm60 selected from early flowering inbreds and the one selected from late flowering inbreds (figure 1 below) and the cross Zm61xZm21 selected from early and late flowering inbreds (figure 2 below).

Bands numbered from 1 to 10, as we can observe that the

cross of early flowering inbred distinguished oneself on the late flowering inbred by appearing new DNA band (number 2) as well as one largest band (number 4), and third band (number 7), which is less clear of them, besides the band number 6 was decayed in the cross of the early flowering inbred. The cross of early flowering inbred superior the cross of late flowering significantly in grain yield, which could be perhaps due to the gene actions of the these three new bands (figure 1 below).

The differ in the number of the size of DNA bands of the cross Zm61xZm21 appeared as a result of PCR test using primer OPE-13 is shown in figure 2. In the ten identified bands, it is noted that the cross of the early flowering inbred differed from the late inbred in decaying band number one in the early inbred and appearing new two bands (bands 2 and 8), which weren't clear in the cross of the late flowering inbred. The dimension and the degree of the clarity of some bands of both crosses can be noted as well, but they didn't differ significantly in grain yield, but the cross of late flowering inbred gave the highest grain yield among the other crosses of the experiment.

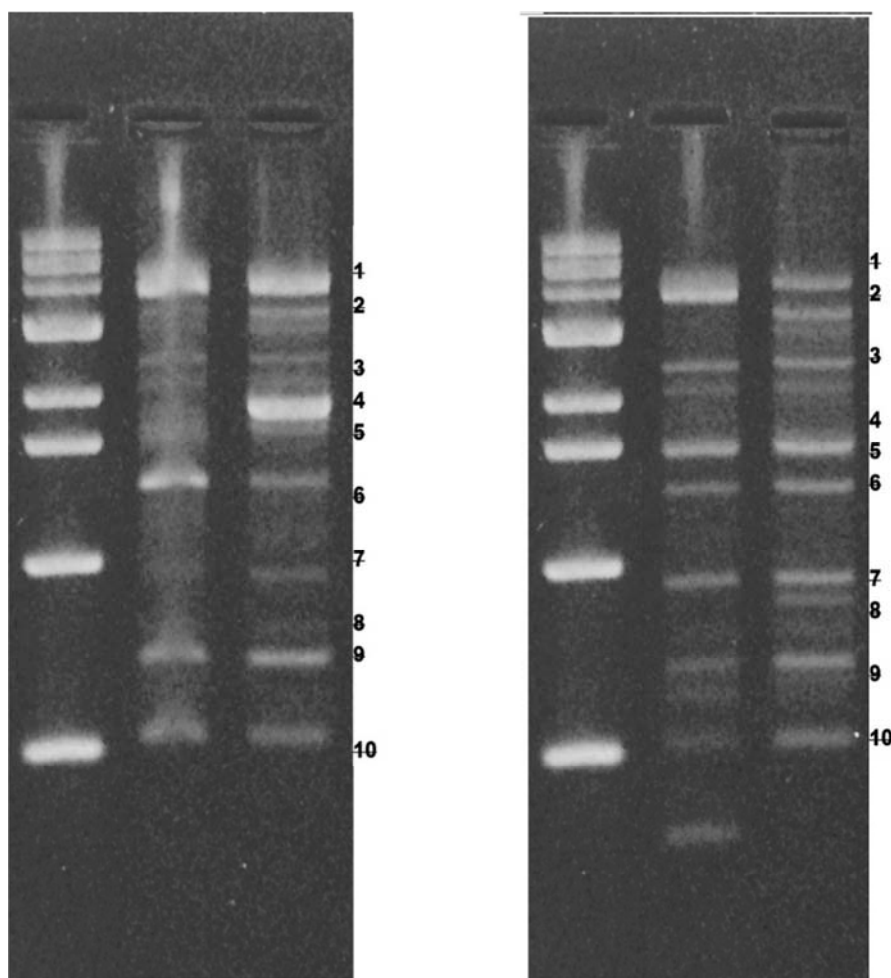


Figure 1. Cross Zm19xZm60 to the right of the reader with the early inbred, in the middle with the late inbred and to the left of the reader the control marker using the primer OPE-13.t (Left).

Figure 2. Cross Zm61xZm21 to the right of the reader with the early inbred, in the middle with the late inbred and to the left of the reader the control marker using the primer OPE-13. (Right).

4. Conclusions

The role of early and late selection on the inbreds was positive is giving crosses of high grain yield, this was due the increasing Days of kernel filling and the high kernel growth rate for some crosses. The growth rate, weight of kernel, and the dry matters assimilation duration of the distinct crosses played significant role in the superiority of the crosses in the grain yield. The Effectiveness of the selection on such inbreds will broaden the genetics base for the breeder as well as increasing the genetic divergence between the mate parents.

Abbreviations

DKF- Days of Kernel Filling; KGR- Kernel Growth Rate; siRNA- small intervening RNA.

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