EPIGENOMICS OF MAIZE HYBRIDS WITH REFERENCE TO KERNEL WEIGHT

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REVIEW ARTICLE

ABSTRACT

Hybrid vigour is one of the most important phenomena became in practice all over the world for its higher performance in maize and several other genera of crops, vegetables, and fruit crops. There is no single theory explains this phenomenon. The only general rule known about having hybrid vigour is that crossing between two genetically diversed inbreds. Epigenomics is thought to be involved in hybrid vigour. However, exact timing of maternal and paternal genomes after fertilization of the endosperm is still not totally understood. It is still believed that the ratio of maternal to paternal gene expression through endosperm development is 2m:1p. The diverged methylation level of the crossed inbreds could be involved in hybrids of maize, at least in part.

Key words*: Zea maize* L., grains, inbreeds, gene expression.

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فوق الجينوم في هجن الذرة الصفراء مع اعتبار لوزن الحبة

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مقالة مرجعية

المستخلص

ان قوة الهجين ظاهرة هامة اصبحت مطلقة على كثير من محاصيل الحقل والخضر والفاكهة ، وذلك لادائها العالي في الحاصل او النوعية ، ولا توجد نظرية واحدة تفسر هذه الظاهرة. اما القاعدة العامة للحصول على قوة هجين هي في تزاوج ابوين او اكثر متباعدين وراثيا. يعتقد ان فوق الوراثة لها علاقة بظهور قوة الهجين . إن التوقيت الدقيق للتعبير الجيني من الاباء والامهات بعد الاخصاب في التأثير في سويداء الحبوب لايزال غير مفهوم، لكنه لايزال المعتفقد عموماً ان مشاركة التعبير الجيني من الام والاب في السويداء هي بنسبة 2 : 1 . يعتقد كذلك ان اختلاف درجة الميثلة في الاباء المتزاوجة قد يكون لها دور في اظهار فوق الهجين ولو جزئياً.

الكلمات المفتاحية: الذرة الصفراء، حاصل الحبوب، سلالة نقية، التباعد الوراثي.

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INTRODUCTION

The kernel weight in cereals is the most important part for nutrients to the developing seedling, and it is also, an important grain yield component beside number of kernels of plant or of a unit of land area. Liu et al (23) reported that 5 pairs of genes control kernel weight in maize (*Zea mays* L.). If this is the case in all maize cultivars or inbreds, or close to that, it implies that the trait is under an additive gene action which means that selection on the inbreds for heavier kernel weight for few generations should enhance this trait to be reflected in its expected hybrid performance. Tollinaar and Lee (27) reported that hybrid maize grain yield in the U.S.A increased about 118 kg/ha/yr since 1930s to the year of 2000, and with about 90 kg/ha/yr in Canada through the same period. That increase was due to two major avenues, enhancement of breeding strategies and improving soil and crop management practices. Growing high grain yield hybrids requires appropriate population densities, weed control, high fertilizer rates, and frequent irrigation. Improvement of a new high grain yield maize hybrid was not a result of improving one trait. There were many plant traits could be improved, such as, higher plant leaf area and photosynthesis efficiency, shade tolerance, higher TDM, longer time for seed-set and filling, stay green leaves, higher tolerance to biotic and abiotic stresses, and higher number of kernels in a unit of land. New maize hybrids reach flowering earlier will have better chance to lengthen time from fertilization to maturity, this will act on two positive traits, heavier kernel weight and better seed-set to have higher kernel number per unit of area. In general, the foregoing traits were not absolutely independent of other plant traits. Higher response of hybrids to higher planting population density, longer seed filling time, one single effective ear per plant, short period from tasseling to silking, late senescence of leaves, higher harvest index, less sensitive hybrid leaves to light quality and duration, better response to frequent irrigation, higher rates of fertilization, and some other traits, all were working together to have higher grain yield of new maize hybrids in a unit of area.

LITERATURE REVIEW

Breeding Inbreds

Maize inbreds bred years ago were of lower grain yield and higher DNA-methylation (14, 15). Todays, maize hybrids are of higher grain yield per plant but lower hybrid vigour. However, these new maize inbreds produce hybrids of higher grain yield. When we realize that kernel weight is under additive gene action, it is easy to select and develop new inbreds of higher kernel weight coincided with higher plant kernel number. This is not difficult in a population of high genetic variations and additive gene action. Some maize inbreds give grain yields close to some low grain yield hybrids. Such inbreds of high specific combining ability are expected to produce elite hybrids, although they could be of lower hybrid vigour than these hybrids developed of inbreds of low grain yield and higher hybrid vigour.

Genome and Environment

Distribution of plant genes and species are related to the variables of environment. The relationship between nature and nurture is important for living beings, humans, animals, and plants. Probably, the most important and clear example of that is the monozygotic twins living in two different environments. This will lead to the important topic in genome and environment, that is the genotype × environment interaction. Bressman and Zhu (6) stated that DNA remains constant from generation to the next. Accordingly, the difference is phenotype counts on the difference in mRNA transcribed from DNA, which is considered as cell-specific gene expression. This is why cells remain similar when propagate via mitosis, while could be different when propagate via meiosis. How genes carry their own memory from generation to the next is not really clear yet. Bressman and Zhu (6) reported that DNA methyltransferase had an important role in epigenetics and what transposable elements (TE) play in that mechanism. Transposons, transcription factors, and repeated elements are thought to be involved in epigenetics mechanism. It is believed that DNA methylation and/or histone modification could be transferred to the next generations without changing DNA sequences or number of

nucleotides. This will leave the epigenetic mechanism effects in action across generations even if they are not seen! How can genes keep their memory from generation to generation without losing control? Sporophytes and gametophytes have different changing process in cell division and growth. If breeders can control gene action, they should be able to have an elephant or a camel from a fertilized egg of a mouse! The route that geneticists and breeders can go through is to treat individuals, watch and select. In human cells, there are about three million of transposable elements, and they are into two types: retrotransposons and DNA transposons, the second not much known about, but retrotransposons can divide and take a position on a chromosome this will change the living being phenotype. In many cases in the plant kingdom, leaves, roots, tubers and stems can be grown to give a normal plant. This is believed to be influenced by a way or another with epigenetics coincided with action of some plant hormones, such as, cytokinen, indoleacetic acid and auxins that control transcription along with siRNA, miRNA or RNAi. All these actions and mechanisms if they are understood, researchers can make much more progress in their project goals.

Cytoplasm in Reciprocal Crosses:

When reciprocal crosses done between every two inbreds, the two resulting hybrids could be different in expressing genes and phenotype. Zhang et al. (32) studied genetic imprinting in maize of reciprocal crosses of B73 and Mo17. They found that the relative expression of the maternal and paternal alleles differs at least five-folds in both reciprocal crosses. They also found that 179 genes of protein coding expressed in the endosperm were transcribed in either sense or antisense orientation from intronic regions, among them 25 maternal and 13 paternal. All differentially methylated regions (DMRs) identified were uniformly hypomethy-lated in maternal alleles, and hypermethylated in paternal alleles regardless of the imprinting direction of their corresponding loci. They concluded that the highly extensive and complex regulation of epigenetic mechanism in maize endosperm can function in the balancing of the gene dosage of the endosperm triploid tissue. On the other hand, Brannan and Bartolomei (5) reported that the imprinted genes found in human and mouse were shown to fall in clusters in the genome. However, Zhang et al. (32) when they dealed with the large number of imprinted genes they found in maize endosperm, that guided them to test those genes whether they were clustered similarly to human, and they found that those imprinted genes and noncoding RNAs fall into 38 clusters. Some clusters were located to gene-rich reigns, and some to gene-poor regions, or average gene density region. At the same time, 13 of 38 genes imprinted noncoding genes were spread in 13 clusters.

Kernel Endosperm and Genomic Imprinting:

As it is well-known today, genomic imprinting is a classic epigenetic phenomenon and it was first identified in maize. It arises from allele-specific epigenetic modifications in which believed to be established during gametogenesis of plant. Dong et al. (9) stated that genomic imprinting in flowering plants occurs primarily in the endosperm, which provides the energy source of embryos and the developing seedling. Several hundreds of imprinted genes have already been identified in a number of genera, such as Arabidopsis, Oryza, Zea, Sorghum, and Ricinus. One of the major questions on imprinting in cereal kernel endosperm is how the allele specific epigenetics modifications are established to determine the parent-of-origin dependent expression of imprinting genes. DNA methylation is an important epigenetic modification involved in regulating gene expression. DNA methylation plays an important role in the allele-specific expression of maternally expressed genes (MEGs) and paternally expressed imprinted genes (PEGs) are shown to be regulated by the DNA methylation glycosylase and/or DNA methyltransferase. Many imprinted genes are associated with the differentially methylated regions (DMRs) where the maternal alleles are hypomethylated and the paternal alleles are hypermethylated. The modifications of histones represent another level of epigenetic modifications. Genome-wide analyses have shown that many types of histone modifications are associated with the

expression or repression of genes in many plants. Dong et al. (9) found allele specific profiles of H3K4me3 and H3K36me3 modifications on a genome-wide scale in maize endosperm at 12 DAP (days after pollination), and that was associated with both imprinted protein-coding genes and imprinted noncoding RNAs. They have concluded that allele-specific active histone modifications (H3K4me3 and H3K36me3) with allele-specific DNA methylation, and repressive modification (H3K27me3) provided a unique look to the regulation of genomic imprinting in maize. When they used histone H3 lysine 4 trimethylation (H3K36me3) antibodies, H3K4me3 modifications were generally restricted to the transcription start sites (TSS), and H3K36me3 modifications were accumulated to high levels in gene bodies, and both H3K4me3 and H3K36me3 showed positive correlation with transcriptional levels in maize endosperm. They used samples isolated from 12 DAP hybrid endosperm tissues from both reciprocal crosses Mo17×B73 and B73×M017, and a total of 54 MEGs and 90 PEGs were identified. That was when the expression level of active alleles at least five times that of silenced alleles.

Imprinting and methylation of MEGs and PEGs:

Endosperm in grain crops is the most important part for nutrients to the embryo and the developing seedling. Beside that, kernel weight and number of plant kernels are two important grain yield components. Xin et al. (29) have studied maize whole kernel and endosperm of kernel transcriptome in reciprocal crosses of Mo17 and B73. Transcriptome sequencing of whole kernel of maize was studied at 0,3 and 5 DAP and the endosperm at 0,7,10 and 15 DAP of the famous USA maize hybrid B73×M017 and its reciprocal cross. They observed a gradual increase expression of paternal transcripts in 3 and 5 DAP kernels. In 7 DAP endosperm, the majority of the gene tested reached a 2:1 maternal versus paternal ratio, suggesting that paternal genes are newly fully activated by 7 DAP. Meanwhile, a total of 116,234 and 63 genes exhibiting parent-specific expression were identified at 7, 10, and 15 DAP, respectively. The largest proportion of parentally expressed genes was at 7 DAP mainly due to the significantly deviated parental allele expression ratio of these genes at this stage, while nearly 80% of the maternally expressed genes (MEGs) were specific to 10 DAP and were primarily attributed to sharply increased expression levels compared with the other stages. It was suggested that 10 DAP endosperm specific MEGs are involved in nutrient uptake and allocation and the auxin signaling pathway, coincided with the onset of starch and storage protein accumulation.

Endosperm Genome After Fertilization:

Although there are thousands of articles published on this topic, but it still that the available and clear-cut information of the timing and extent of activity of maternal and paternal genomes after fertilization is limited and not in full agreement. However, results obtained by Nodine and Bartel (24) on maize kernels suggested that the activation of paternal and maternal alleles could be relatively delayed in the embryo, although contrary evidence indicated that the two sets of parental alleles are activated simultaneously. Xin et al (29) found that at 3 DAP, 941 genes were shown to produce paternal transcripts less than the maternal transcripts. Of the 941 genes, 923 were also expressed bias may not necessarily be a reflection of a delay in paternal genome activation but rather due to expression or presence of the transcripts in the maternal kernel tissue. So, it is reasonable to conclude that paternal genome activation was nearly fully achieved by 7 DAP or earlier because the majority of the 11027 genes with SNPs were shown to be biallelically expressed, and the proportion of the expressed genes with the expected 2m:1p ratio was similar in 7 DAP endosperms.The paternal genome tested after 3 and 5 DAP of maize hybrid kernels, plotting the paternal versus maternal expression of all retained genes have explained a dual activation of the paternal genome in 3 and 5 DAP kernels in terms of both the numbers of activated genes and their expression levels. The number of genes with activated parental alleles increased from zero at 0 DAP to 941 at 3 DAP, and then to 4063 at 5 DAP. In 7 DAP endosperm, 11027 genes containing SNPs were expressed, 8128

(73.71%) exhibited the expected 2m: 1p ratio, whereas 2899 (26.29%) genes exhibited allele-biased expression. Similar proportions were observed in 10 DAP endosperms, with 72.75% (7691) of the genes exhibiting the expected ratio and 27.25% (2882) exhibited an allelic bias. This implies that complete transcriptional activation of the paternal genome was achieved by 7 DAP in the endosperm (29).

Transcriptome in the Reciprocals:

Some relatively recent researchers have indicated that transcriptional activation of the two parental genomes might not occur at the same time in the zygote during early kernel development (16). A more recent article by Nodine and Bartel (24) reported that both the paternal and maternal genomes might contribute equally in early stages of embryonic development. However, timing and proportion of paternal and maternal genome activity during early endosperm development at least in maize kernels is still not clear. Zhang et al (32) have expected that only a small number of imprinted genes have been identified in developing embryo and endosperm of maize kernel. Looking at the genome assembly of inbred M017 based on inbred B73 genome, Xin et al (29) found that genome of M017 was 2, 058, 527, 894 bases! containing a total of 117, 847, 390 bp short gaps. Thus, the coverage of the M017 genome was in 94.3% producing a similar genome size to the 2,066,433,971 bases of the inbred B73 reference. The identification of single nucleotide polymorphisms (SNPs) and insertions and deletions were performed, and found that there were 6,557,611 SNPs identified, 157,994 insertions, and 194,549 deletions between inbreds B73 and M017 genomes.

SNPs and Methylation of MEGs and PEGs:

The basic reasons for hybrid vigour are still not clear, but researchers still believe that the highly genetically diverged parents are more likely to be candidates to produce elite F1 hybrids. However, number of genes or SNPs involved in this phenomenon is not constant. Grossmann et al. (17) stated that in several cases, epigenetics and epialleles are involved in hybrid vigour. sRNA of 24 nt has an important role if its number in the crossed parents is different. They attributed that to differential DNA methylation between parents and this F1 hybrid. RNA directed DNA methylation is of a special effect of differential methylation between crossed parents and this F1 hybrid causing less cytosine methylation. sRAN of 21-24 nt supposed to play an important route in epigenetic mechanism. Barber et al. (3) studied the inbreds B73 and M017, and their reciprocal crosses and confirmed that the F1 hybrid had less number of 24-nt siRNA than the parental inbreds. sRNA is known today to control gene action and conserve the genome from generation to next. Less number of 24-nt siRNA in hybrids is the reason of lower methylation in the resulting hybrid as compared to its parental inbreds. RNA dependent RNA polymerase 2, along with some other hormones and enzymes, epigenetic mechanism will be involved even if it is not really seen. On the other hand, Rigal et al (25) when they studied F1 hybrid of A. thaliana found that when methylation in the hybrid goes down, many genic expressions appear and not exist in its parental inbreds. They explained that when two groups of genomes diverged in methylation levels, the epigenomic shock will take place causing that vigour. Those actions were attributed to transposon activities in the united cells in the hybrid individuals. On the other hand, Birchler et al (4) explained the reason behind hybrid vigour to series of alleles such as A1, A2 and B1,B2 that complement each other in the hybrid with series of those alleles. The whole maize kernel imprinted genes were analyzed by Xin et al. (29) in their study on both reciprocal crosses of B73xM017. They found 11,027910,573 imprinted genes, and 7777 SNP containing genes in the 7, 10 and 15 DAP endosperms, respectively. The genes exhibiting parent – specific patterns in both reciprocal crosses were selected as candidate imprinted genes, and found that 284,606 and 190 genes were determined to have allele-biased, parent-of-origin specific expression patterns at 7,10 and 15 DAP, respectively. There were 300 PEGs and 499 MEGs identified from the three developing endosperm stages. Among the 499 MEGs, 418 were identified at 10 DAP only, and only 15 were identified at all three time points. Meanwhile, among the 300 PEGs, 213,130,

and 163 were observed at 7,10, and 15 DAP, respectively.Waters et al (28) reported that inbred-line dependent imprinted genes that exhibit allele-specific expression was in one direction of each reciprocal pair of crosses, but biallelic expression was in the other direction. Xin et al (29) reported that two more types of allele-specific expressed genes that have allele-specific expression is widespread and believed to be responsible for quantitative variations in the phenotypic traits of hybrid offspring. In the three staged samples of endosperm 1688 B73 allele-specific and 1130 M017 allele-specific genes were detected, that exhibited expression dosages deviating from the 2m:1p ratio. Among these, 303 B73 genes and 128 M017 genes exhibited a non-parent-specific, monoallelic expression pattern in both reciprocal crosses.

MEGs and PEGs Pattern of Imprinting Expression in The Endosperm:

Xin et al. (29) in their study on B73 X M017 reciprocal crosses have identified 4 groups out of 290 imprinted genes they identified. These groups included: 1. Regulation of gene expression by genetic imprinting, 2. Endosperm development, 3. Response to hormone stimulus, and 4. DNA-binding. These functional categories indicated involvement of the imprinted genes in hormone signaling pathways and transcriptional regulation of endosperm development. When the 194 MEGs and 96 PEGs were examined separately, they found only two categories and one category exhibiting significant enrichments for MEGs and PEGs, repetitively. The first category of MEGs was the: response to hormone stimulus which included 13 genes, 6 of which were involved in the auxin-mediated transcriptional responses. The second was the cytoplasmic membrane-bounded vesicle, which included 26 genes encoding a variety of enzymes, transporter proteins, and cell wall formation. Proteins located in the cytoplasmic membrane, suggesting that these MEGs might be involved in intercellular nutrient transport and signal transduction. The only one enriched among the 24 PEGs was the binding category. This category represented various forms of molecular interactions, such as, DNA binding, RNA binding, protein binding, and ATP binding. However, the functions of MEGs and PEGs are not known!. The same researchers found that MEGs and PEGs exhibited distinct patterns of gene imprinting during endosperm development, with PEGs predominately detected at 7 DAP MEGs predominately detected at 10 DAP. Out of the 194 MEGs identified in the three stages, 150 (78.5%) were shown to be expressed maternally in both crosses uniquely at 10 DAP. On the other hand. The 7 DAP endosperm contained the highest number of PEGs (80 out of 96), 26 of which were shown to be paternally expressed only at this developmental stage in both crosses. Meanwhile, they found the occurrence of 7 DAP specific PEGs were mainly due to the significantly deviated paternal allele ratio, whereas, the 10 DAP specific MEGs could be primarily attributed to the sharply increased expression levels at the respective stages. Three 7 DAP specific PEGs and nine 10 DAP specific MEGs were studied, all the three PEGs exhibited paternally biased expression patterns at 7 DAP in both reciprocal crosses, although their maternal alleles were strongly expressed in M017 x B73 crosses. All nine MEGs tested showed a predominant pattern of expression at 7 or 15 DAP in both reciprocal crosses. Whereas, among the 10 DAP specific MEGs, two were identified to encode auxin response factors belong to the auxin-indole-3-acetic acid family. These results suggest that auxin-mediated nutrient uptake from maternal tissues to the endosperm is under control of the maternal genome.

Epigenomic Mechanisms in Maize Hybrids:

Hybrid vigour in maize and many other crops; fruit trees, and vegetables was applied for years without one theory explains this phenomenon. Epigenetics or epigenomics is referred to the biochemical changes in nuclear DNA, post-translational modifications in histone proteins and variations in the biogenesis of small non-coding RNAs in the cell. These changes are often responsible for variation in gene expression without a change in the base pairs of nucleotide sequence. This kind of changes may cause variation in the chromatin structure leading to changes in activity of gene expression. Maize is the most studied crop in terms of genome, epigenome and hybrid vigour. Many researchers have shown that epigenetics has been shown to play

an important role in hybrid vigour in maize hybrids and other crop hybrids (3, 9, 12, 13, 14, 15, 18, 21, 22, 27). Meanwhile, Bressman and Zhu (6) stated that in two years only, 14,000 articles were published on epigenetics!. Dong et al. (9) stated that genomic imprinting is a classical epigenetic phenomenon identified first in maize, and it arises from allele-specific epigenetic modifications that are generally believed to be established during gametogenesis. Elsahookie (12) reported that epigenetics is supposed to occur through meiosis and mitosis. In flowering plants, genomic imprinting takes place mainly in the endosperm of cereal kernels, that is the main source of energy to embryo, and the developing seedling. Elsahookie (13) reported three mechanisms of genomic imprinting, DNA methylation, histone modification, and RNA interference. Most likely, DNA methylation is the most clear pathway in epigenomics in plants. Nonallelic expression of some sets of genes depends on paternal and/or maternal effects is supposed to be regulated by DNA methylation. Endosperms and embryos of hybrid maize kernels showed some differentiated results. Regal et al. (25) stated that genes and transposons can exist in variable DNA methylation states, with potentially differential transcription. Novel nonparental and heritable epialleles arise at many genic loci in the hybrid, and they suggested that combining distinct epigenomes of two parents can create novel patterns of genes and transposons regulation. Meanwhile, Inoue et al. (19) revealed that a few genomic loci are maternally imprinted because of the inheritance of maternal histone 3 lysine 27 trimethylation (H3K27me3). They also stated that DNA methylation in plants occur in three cytosine contexts; CG,CHG, and CHH, where H is either A or T or C. On the other hand, they have used cells from immature maize kernel embryos (12 DAP) in tissue culture test, and found that the correlation between 21-24 nt small RNA and DNA methylation regions was correlated most with 24 nt small RNA. Meanwhile, Lauss et al. (21) in their work on Arabidopsis hybrids found that several strong positive and negative heterotic phenotypes among what they called epiHybrids, indicating that epialleles were acting. Hanna and Kelsey (18) reported that monoallelic expression of imprinted genes is predominantly controlled by DNA methylation inherited from the parental germ cells, and the questions of whether epigenetic modifications other than DNA methylation, such as histone marks are transmitted from gametes, and whether they are capable of mediating imprinted gene expression remains outstanding. On the contrary, Lauss et al. (21) reported that nuclear, extra-nuclear, and epigenetic role in hybrid vigour is still unclear. On the influence of environment (abiotic stress) on adaptation and epigenetic mechanism in plants. (11, 12, 13) reported that environment has an effect on plants living under abiotic stress, and that could create a new phenotypes of novel genes that can be transmitted to next generations. This idea has been confirmed later by Zheng et al. (33) when they stated that drought adaptability of rice plant has been improved because of multi-generational drought exposure. They identified appearance of some drought induced epimutations could maintain the altered DNA methylation level in the subsequent generations. Analyzing of the drought-associated genes revealed that the DNA methylation level of the genes was affected by the multi-generational drought at stress. These results suggest that epigenetic mechanism plays an important role in plant adaptation and/or evolution, at least in some specific conditions, such as salinity and drought stresses.

Kernel Traits of Maize Sub-Species Crosses:

Chen et al. (7) reported that kernel weight in newer maize hybrids have 15-23% increase and an average of 150 kernel/m^2 as compared to old hybrids. In earlier study, Chen et al (8) stated that new maize hybrids are higher in grain yield than old hybrids by 1.3-3.1 t/ha, and that due to high nitrogen content in new hybrid leaves at maturity. This implies that stay-green hybrids and/or inbreds are more promising in grain yield than other genetic materials without these traits. It is well known today that new maize hybrids are responding more to higher rates of fertilizations, frequent irrigation, and higher planting population density. This means that newer maize hybrids are less sensitive to light intensity and

duration. These traits are so important in new hybrids to give higher grain yield. Management practices are the second important variable that complement the higher grain yield in maize hybrids. This includes favorable planting population density, favorable rates of fertilization, frequent irrigation, and better soil ploughing and disking. Tollernear and Lee (27) reported that the average annual grain yield increase of maize in the U.S.A is about 118 kg/ha, and that about 65% of that increase is due to genetic improvement. Some researchers recommended using erect or semi-erect leaf genotypes of maize, but to my understanding through my work on maize for about fifty years, I found that this trait is not necessarily to be always true. We have been dealing in teaching genetics and breeding that DNA is double-helix, while Zeraati et al. (31) found that DNA in human cells is tetra-helix. That was found in twisted "knot" of DNA, called it i-motif, never been seen before in living human cells. This is an example that many of our ideas in science could be changed due to new discoveries. We have reported that results of Xin et al. (29) on gene expression of maize hybrid kernels, and some of these results that expression of paternal genes was clear at 3-5 DAP, and that expression extends as the kernel develops, while maternal to paternal gene expression in endosperm was 2 m:1p at 7 DAP. Meanwhile, they have identified 116,234, and 63 paternal gene expression at 7, 10, and 15 DAP, while maternal gene expression reached 80% in the endosperm at 10 DAP. However, they showed that it was so difficult to identify the complete picture of maternal and paternal gene expression in embryo and endosperm in that detailed study of the reciprocals crosses of maize hybrid B73 x M017 and M017 x B73, for the complications of several processes at different stages of kernel development. Previously, Sabelli and Larkins (26) found that endosperm in grasses start fast cell division 4-5 DAP and continues up to 8-12 DAP, and it could proceed to 20-25 DAP, especially at the peripheral region of endosperm. Nonetheless, the final outcome of kernel and embryo is well known, while the perfect or exact gene expression of both maternal and paternal is not known yet.

In practice, several crosses have been produced and evaluated in the field on some sub-species of maize genotypes. Elsahookie (10) used four different maize sub-species, namely, Zm2 (flint) *Zea mays* L., indurata, Zm19 (dent), indentata, P8 (popcorn, everta), and sweet-corn (S) saccharata. These genotypes were crossed and traits of F1 kernels are shown in Table 1. The symbols 2 for Zm2, 19 for Zm19, P for P8, and S for sweetcorn were used for simplicity. Table1 shows that kernel weight of the cross 2×19 was close to the maternal parent, and some trend in its reciprocal cross 19×2, while 2×S shows the effect of maternal cross S×2 which gave clear overdominance in kernel weight (464 mg as compared with parents 206, and 306 mgs). However, crosses of 2×P and P×2 gave less kernel weights than this parent, that was negative hybrid vigour. The cross 19×S gave kernel weight similar to maternal parent,

Table 1. Means of traits of F1 grains of parents and their crosses of some maize sub-species (from Elsahookie, 10).

Parents and crosses	mg kernel wt.	Kernel shape	Kernel colour	Dent shape
Zm2	306	Round(R)	Dark yellow (DY)	Flint (F)
Zm19	345	Triangular(T)	Light yellow (LY)	Dent (D)
S	206	Shrunken(Sh)	DY	Shrunken
P	320	Tipped round(TR)	LY	F
2×19	296	R	DY	F
19×2	338	midparent	Midparent	D
2×6	320	R	DY	F
S×2	464	R	LY	D
2×P	264	R	DY	F
P×2	238	midparent	DY	F
19×S	340	T	LY	midparent
S×19	410	midparent	LY	D
19×P	338	T	DY	D
P×19	316	midparent	DY	F
S×P	364	T	LY	D
P×S	320	midparent	LY	F

while S×19 gave kernel weight of 410 mgs, which is again a positive hybrid vigour. Another impressing kernel weight vigour was in the cross S×P, while its reciprocal cross P×S gave similar kernel weight to the maternal parent. The conclusion of these results is that there is no consistent rule to inherit kernel weight, although in many cases, the maternal effect is common, but the plant breeder needs to test those crosses and find which cross is better in giving higher kernel weight. These kernels were F1 kernels, and we have no idea what will happen to F2 seeds, which they are the commercial seed that make farmers to by F1 seeds to have higher grain yield of F2 seeds on F1 plants. Kernel shape, kernel colour, and dent shape of kernels can be observed from same table.The results of Table1 gave us a question, that is: what will the F2 kernels look like in weight?, Yousif and Elsahookie (30) examined similar maize sub-species crosses, but proceeded to the F2 seeds. Before we go to the kernel weight of F1 seed, let us take a look at Table 2 which shows some traits of the ear of maize of parent and their crosses in both ways, with an extra parent (D). Data of Table 2 shows that there was maternal or paternal effect on traits of ear on maternal plants due to crossing. There were positive, negative hybrid vigour or mid-parent values of several traits of maize ears. That could be due to xenia effect, and/or dominance or hybrid vigour, positive or negative without a well-known explanation. So, let us go now to Table 3 of F1 and F2 seeds. Results shows in Table 3 clear increases in kernel weight in F2 seed than F1 seed, very rare crosses gave little increase, but most of other crosses gave higher kernel weight of F2 seeds carried on F1 plants. Another important comparison is that of kernel weight of F2 seed as compared to both parents. Almost, all F2 kernel weights of crosses were higher than kernel weights of their parents, except one case of P×19 which was male sterile. An idea come to my mind to test some divergent traits of some inbred, such as early versus late flowering plants. Since, it was not easy to have double haploid inbreds because of lack of facilities, the inbred populations still have some variations in plant height, time of flowering, pigments on tassel and/or silk, time between tasseling and silking, etc. The trait that was chosen on some inbred population was very early few plants and very late few plants, selfed, and crossed to other genetic material, and this was some of the work of Al-Khazaali et al.(1, 2) (Table4). Four selects were taken from inbred populations (early and late flowering), they were 19,32,51 and 61 inbreds used as females. Two testers were used; inbreds 21 and 60. Data of Table 4 show that early and late selects from same inbred populations are not necessarily to be small or large kernels all the way. Crosses of early and late selected inbreds with same tester gave different kernel size. However, most crosses were not significantly different in kernel weights of early and late selects, except the cross 32×60 which gave a significant difference at 10% probability level, and it was close to be significant at 5% probability level. Meanwhile, DSF among early and late crosses had significant differences in seven crosses, and only the cross 51×21 gave the same value. Grain yield is the outcome of these major components, grain weight, grain per plant, and total number of grain per unit of land area. However, these three components are always related to other sub- components such as seed growth rate, harvest index, seed filling period, and probably some other variables. The most prominent result in Table 4 was the grain yield of the cross 19×60 which gave 10.52 and 8.19 t/ha for early versus late flowering selected inbreds. It was suggested to take more divergent traits of inbreds populations to be tested for grain yield and other traits. Elsahookie et al.(14, 15) tried same idea to select some plants different in some traits of the same inbred population, selfed, crossed with a tester, then test the performance. Table 5 shows the most important results they obtained. Results in Table 5 indicate that selection for a specific phenotypic trait among inbred population could be positive or negative. The letters used with some inbred numbers such as, fw, dw, dr, and sg pertained to flent white cob, dent white cob, dent kernel of red cob, and stay-gray leaves, respectively. There were two higher grain yield of some selected inbreds, those of crosses 60×73fr and 60×73dr which gave 11.10 and 11.15 t/ha, respectively.

Table 2. Some traits of ear of parents and their crosses of some sub-species of maize genotypes* (F1 seeds on maternal plants).

Parents (crosses)	ear length cm	rows/ear	ear/plant	kernel/plant	grain(g)/plant
D	20	14	1.8	647	193
P	21	16	2.4	1436	262
S	19	18	1.2	582	108
19	16	18	1.4	621	119
2	16	17	1.4	450	92
D×S	21	18	1.0	657	214
S×D	20	17	1.4	650	177
D×P	21	17	2.0	626	220
P×D	17	13	1.2	578	148
D×19	14	14	1.0	605	210
19×D	20	14	1.2	933	274
P×S	18	16	1.2	536	185
S×P	20	17	1.4	673	170
P×19	16	16	2.5	1573	357
19×P	20	19	1.0	712	249
P×2	16	16	2.0	600	139
2×P	19	17	1.0	615	175
S×19	22	15	1.2	638	211
19×S	20	14	1.2	505	202
S×2	22	16	1.5	1008	175
2×S	23	16	1.2	487	144
19×2	22	17	1.0	575	187
2×19	22	16	1.0	555	171

*** Plants were grown in a population density around 40,000 plants/ha.**

Table 3. Some kernel traits of some sub-species of maize crosses in F1 and F2 kernels.

Parents	Kernel wt.mg		embryo wt.mg		% of embryo to endosperm	
D	294		41		16	
S	114		16		17	
P	162		22		16	
2	232		40		21	
19	200		28		16	
crosses	F1	F2	F1	F2	F1	F2
D×S	288	341	55	62	24	22
S×D	160	260	44	59	38	29
D×P	320	385	62	70	24	22
P×D	171	251	30	38	21	20
D×19	298	325	52	55	21	20
19×D	255	280	46	48	22	21
P×S	165	318	31	55	23	21
S×P	185	253	30	40	19	19
P×2	140	240	27	38	24	19
2×P	151	292	23	41	18	16
P×19	160	sterile	26	sterile	20	sterile
19×P	328	357	50	53	18	17
2×19	228	307	48	53	26	21
19×2	196	232	31	35	19	18
S×19	280	312	49	50	21	19
19×S	235	344	51	63	28	22
S×2	285	298	29	43	11	17
2×S	118	287	17	40	17	16

Table 4. Kernel wt.(mg), days of seed filling (DSF), kernel/m^2 and grain yield of crosses (t/ha) of early and late selected plants from some inbred population.

Crosses	Kernel wt.(mg)		DSF		Kernel/m^2		Grain yield (t/ha)	
	Early	Late	Early	Late	Early	Late	Early	Late
19×21	199	171	38	35	3328	3349	6.64	5.62
32×21	208	191	40	36	3697	3839	7.69	7.37
51×21	250	245	38	38	4037	4056	10.21	9.95
61×21	217	240	40	35	4561	4565	9.90	10.90
19×60	294	191	37	34	3591	4296	10.52	8.19
32×60	244	205	38	35	4232	4513	10.31	9.16
51×60	232	225	38	36	4317	4145	10.03	9.39
61×60	228	215	37	34	4519	4658	10.20	10.02
LSD%5	40		2.0		847		2.06	

Table 5. Some traits of some hybrids tested depending on selection among inbred population. The absolute number of inbred is the original one, and the same number with letters are the selected one S (from Al-Khazaali et al. 1, 2).

Crosses	Leaf area m^2/plant	Row/ear	cm/ear Length	kernel/ear	mg,kernel weight	Grain yield t/ha
60×73fw	0.445	18.8	18.8	678	170	9.23
60×73dw	0.425	17.3	16.3	661	148	7.84
61×73dw	0.428	17.5	16.9	540	181	7.80
61×73dr	0.434	15.8	17.4	424	230	7.40
17A×844	0.486	14.5	16.0	343	284	7.80
17×844	0.496	15.8	15.3	513	245	10.03
74sg×21	0.477	17.8	16.4	552	207	9.15
74sg×73dw	0.578	18.0	17.8	575	223	10.25
74×21	0.480	15.5	17.5	497	230	9.20
60×73fr	0.439	18.3	16.6	713	195	11.10
60×73dr	0.403	17.5	15.9	672	212	11.15
17D×73dr	0.532	16.8	17.1	586	207	9.70
17E×844	0.420	13.8	13.6	335	213	7.40
60×73	0.450	15.5	16.8	501	220	9.15
LSD 5%	0.046	01.8	1.0	107	027	1.70

These two values should be compared with the original two inbreds of the cross 60×73 which produced 9.15 t/ha. The differences in grain yield were significant and remarkably worth. The difference of the two crosses above the original cross is about 2 t/ha, this is an excellent positive value gained due to this relation. This project will be extended, and so far, we have several new selected inbreds to be crossed and field tested for their performance in the next seasons. Hybrid vigour phenomenon is still genetically unknown reason, but it is famous that crossing two or more genetically diverged inbreds could be give an elite hybrid. Epigenomic mechanisms could be involved in this phenomenon. Some of noncoding RNA could be the key of switching genes off and on. Epigenomic mechanisms suggested for hybrid maize kernels were in four categories: 1- regulate gene expression by genetics imprinting, 2- regulate endosperm transcriptional development, 3- control responses to hormone stimuli, and 4- DNA-binding. More studies are required on an extensive level to test field performance, genetic actions, and epigenomic analyses of several crosses of several diverged inbreds. New higher technologies are expected to uncover many new approaches in this phenomenon. However, selection and selfing of some different selects among available inbred populations well be helpful for newer elite maize hybrids. Heavier kernel weight, and higher kernel number of the ear are of prime importance in such selection program.

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