Chapter One
1. Introduction

It may not come as a surprise when we say that maize (*Zea mays* L.) represents an important crop in terms of both use and trade. This irrefutable fact is based on the notion that the crop represents one of the key tools in food security strategies to diminish the gap between global production and consumption of food. Moreover, maize importance has increased due to the great variety of its uses. The crop supplies raw materials for many industries such as plastic, anti-freezing and as bioreactors in producing biopharmaceuticals and as biofuel (Ganapathy, 2016; Hubbs, 2017).

The prestigious position of this crop leads to a continuous work to increase its productivity in the unit area to the maximum possible extent. Even though heterosis is one of the strongest means in a achieving such goal, it has not been evidently interpreted in spite of combination of several classical and molecular hypotheses proposed for its explanation (Jin et al., 2017; Khotyleva et al., 2017).

Molecular markers represent the most important modern technologies that have been adopted and evolved to solve the ambiguous heterosis phenomenon. In the last few years, researchers have made great efforts to settle new molecular markers that enable the categoration of any germplasm into distinct heterotic groups to be more efficient in using heterosis (Larièpe et al., 2017).

Most of the DNA markers expose high genetic differences with corresponding nucleotide sequence (Lee et al., 2007; Qi et al., 2010). This makes it necessary to move up to a higher level of technologies to improve our understanding of the two contradictory concepts, hybrid vigor and inbreeding depression. Epigenetic

has become a talk of the hour and took a great deal of specialist time and effort in an attempt to link it with the hybrid vigor. Epigenetic effects such as DNA methylation are not reliable on the amendment of the DNA nucleotide sequence (Zannas and Chrousos, 2017). In fact, it's a reversible modification relates to the alteration in the chemical affinity between the DNA and the methyl group. The effects of such variations are highlighted by many vital events especially the regulation of genetic expression in hybrid vigor and inbreeding depression even in the absence of genetic diversity (Dapp et al., 2015; Springer and Schmitz, 2017).

The epigenetic effect of methylation is the most distinguished markers that used in many eukaryotes and considerably in plants. The biochemical modification of DNA methylation plays various roles during the different stages of normal differentiation and development, in addition to regulation of gene expression in times of biotic and a biotic stresses (Lukens and Zhan, 2007; Zilberman, 2008; Lu et al., 2017).

Numerous studies reported that the DNA methylation level in maize hybrids could be remodeled prominently compared with their parental lines, which may operate to regulate the additive or the non-additive gene expression in the hybrids (Zhang et al., 2008; Zhao et al., 2007). Definitely, there is a statistically interrelation between special DNA methylation level and heterosis in many plant species, especially maize (Almelhami, 2017; Shen et al., 2017).

Inbreeding leads to sharp decline in the fitness of most naturally outbreeding species. Although, it has been a long time since the first observation of this harmful aspect however the molecular mechanism behind it is not clearly understood (Paige, 2010). There is still an urgent need to find proper answers to many important questions such as; whether there is a stable relationship between the selfing-generations and DNA methylation or not?, and what makes

such kind of relationship in many occasions imperceptible or mysterious, while it could be evidently perceptible in other times.

Accordingly, this study has been projected to investigate the potential role of selfing in the depression of maize inbreds performance and its relationship with DNA methylation level and the hybrid vigor of the first filial generation in half diallel of maize.

Chapter Two

2. Review of Literatures

2-1. Epigenetic Variation

The traditional plant breeding focused on capturing and gathering as many as possible of the variable desired alleles to improve traits in question and make plants more efficient in using limited resources (Postnote, 2017). As previously expected, genetic variation that commonly points to the heritable variation of genetic information found in individuals and populations is lonely responsible for revealing the organism traits (Goulet et al., 2017).

Researchers made great efforts to discover other variations in view of the various traits among individuals that follow the same species, which have no direct correlation with DNA sequence polymorphisms. These newly formed variations that coined "Epigenetic", received a considerable attention for better understanding to its stability through successive generations (Springer and Schmitz, 2017).

The heritable or reversible changes in gene expression happen at a level higher than the level of the nucleotide sequence. In other words, it is not attributed to alterations in the type and/or sequence of DNA nucleotides (McKeown and Spillane, 2014; Lu et al., 2017).

Genetic and environmental variations and their interactions may naturally induce the phenotypic variations. Altered phenotypic traits may be resulting from an identical genetic structure and such alterations resulting from identical alleles acting in different ways in responding to biotic or abiotic stress (Fortes and Gallusci, 2017). The characterized natural epialleles are relatively few, consequently the role of epigenetic variation for revealing big phenotypic alterations is still vague (Springer and Schmitz, 2017).

Epigenetic changes usually participate with a variety of chromatin marks, such as cytosine methylation, modifications of histone tail, chromatin remodeling and non-coding RNAs (Rajewsky et al., 2017).

2-1-1. Histone Acetylation

Nucleosome is the basic unit of chromatin consists of roughly 147nt of DNA wrapped around a core of octamer histone proteins, two copies from each (H2A, H2B, H3 and H4). Histones are highly positive charged proteins, contained within their sequences approximately 24 molecules of lysine and arginine. As DNA can be methylated, histones can be subjected to numerous post-translational modifications (acetylation, methylation, phosphorylation… etc.), (Grabsztunowicz, 2017).

Histone acetylation is the other wide studied form of epigenetic modifications in addition to DNA methylation. In plant biology, acetylation exposed several observation points which prove its importance. It seems obvious that the core histones are reversibly acetylated (Liu et al., 2017), and they play a crucial role in the post-translational modifications, like acetylation, methylation, ubiquitination, phosphorylation and ribosylation of ADP (Berger, 2007). The striking fact is that many of the epigenetic mechanisms operate cooperatively via organizing the work of each other to regulate gene expression during cell differentiation. This "fine-tuning" mode of action guarantees precise operating system, just as in histone deacetylation which helps in the maintenance of DNA methylation (Blevins et al., 2014; Lee et al., 2017).

A few years ago, the reality of DNA methylation is relatively similar to its counterpart in plants at present, the histone acetylation. It should be noted here that the essence of DNA acetylation involves the addition of acetyl ($CH3COO^-$) group to the $NH3^+$ group of lysine amino acid, whereas the histone deacetylation eradicates the acetyl groups (Boycheva et al., 2014). These claims give a strong

argument to those who consider increasing activity of any gene often accompanied with promoted lysine acetylation in the core of histone tails (Xiao et al., 2017). Advancement in epigenetic research enables the understanding of the function and regulation of histone acetylation in the plant more accurately than that assessed by inhibitors which actually do the opposite. Many copies of histone acetyltransferases and histone de-acetyltransferases have been a biochemical characterization in maize (Zhou et al., 2017).

It is not evident that how chromatin structure is modulated by different acetylation of histone. However, the most common scenario is suggested by the crystal structure of nucleosome in which non-acetylated tails of histone are available to interact with nearby nucleosome beads and moderate higher-order chromatin wrapper. Therefore, this is the suggested norm of action of histone deacetylases, while histone acetyltransferases moderating chromatin relaxation (Boycheva et al., 2014; Peng et al., 2017).

The most important histone modification, which obtained greater attention, is the acetylation of protected lysine α-amino acid, particularly in the amino-terminal tails of histone. The competitive effects of either HAT or HAC histone acetyltransferase enzymes against histone deacetylases enzyme (HDAs) determine histone acetylation levels (Ma et al., 2013; Wang et al., 2014). Dozens of HAT, HAC and HDAs have been characterized in plants not only by its critical role as biotic and abiotic stimulants but as functional regulators in the normal development process (Zhao and Zhou, 2012; Peng et al., 2017).

In most eukaryotes like plants, histone acetyltransferase enzymes are classified into two major classes, namely HAT-A and HAT-B (Liu et al., 2017). The HAT-A enzymes have interesting importance since they occupy the nucleus, and operate to acetylate core histones which have been integrated into the chromatin, so they are reversibly involving in controlling of gene expression (Boycheva et al., 2014).

The main changes in histone acetylation were found to be related to DNA replication at the cytological level instead of their relation with transcriptional activity (Vergara and Gutierrez, 2017). Such changes have been identified by using acetylated histone antibodies isoforms (Li et al., 2017).

During cell cycle, there are various oscillations with histone acetylation that are controlled by HAT-B enzymes (Class 2 of histone acetyltransferases). Free cytoplasmic histone (H4 or H3) firstly acetylated by HAT-B enzymes, after that nuclear income and deposition into recently replicated chromatin (Yang et al., 2011). According to single nucleotide homology, plant HDAs have been grouped into three groups, Reduced potassium dependency 3 (Rpd3)-like families and the plant specific histone deacetylase 2 (HD2) are their well-known examples (Ma et al. 2013; Wang et al. 2014).

2-1-2. Non-Coding RNA

The tremendous revolution in the epigenomic era provided researchers with unexpected valuable findings which prompted them to rethink repeatedly. In general, the eukaryotic genome is not simple, and over time we have seen a clear evidence endorse the complexity of such genomes (Jin et al., 2017). Many of the scientific facts that were previously believed to be absolute were brought back to the table of discussion. One of these abolished facts is that the transcriptomes result exclusively from protein-coding domains (Berretta and Morillon, 2009). The surprising finding is that the encoding proteins come from a tiny portion (2–25%) of the total space of genome (Liu et al., 2015).

Although non-coding RNAs (ncRNAs) are not translated into proteins, they are considerable regulators of various biological processes (Liu et al., 2017). In addition, ncRNAs have been revealed to play a pivotal role in the direction of plant growth, differentiation and regulation of plant response to environmental stresses at either transcriptional or post-transcriptional levels (Sunkar et al., 2012; Matsui et al., 2013).

Non-coding RNAs can be classified into two classes, the well studied small ncRNAs (sncRNAs), which consist of less than 200 nt and long ncRNAs (lncRNAs), which is consist of more than 200 nt, and it is less studied compared with sncRNAs (Zhang and Chen, 2017).

Small RNAs (sncRNAs) are non-coding RNAs (ncRNAs) with too small molecular weight, and it has been studied extensively for decades in both plants and animals (Bhatia et al., 2017). Basically, there are two main groups of plants sncRNAs, short interfering RNAs (siRNAs) and microRNAs (miRNAs). The two groups are different in their genetic origin, and their final consequence of regulation (Rajewsky et al., 2017).

Typically, siRNA refers to exogenous double-stranded RNA (dsRNA) that is brought from out of the cells, whereas miRNA is single stranded result from endogenous stem-loop non-coding RNA (Guleria et al., 2011). Each of non-coding RNAs, siRNA and miRNA are processed inside the cell by the RNase Dicer-Like, then they will transported out of the nucleus to bound with Argonaute (AGO) proteins into the cytoplasm and integrated to shape the RNA-induced silencing complex (RISC), (Prathiba et al., 2017) which in many occasions regulates the expression of the target gene at the post-transcriptional level (Vaucheret et al., 2006; Carthew and Sontheimer, 2009). The mobility of sncRNAs molecules inside the organism may serve to ease gene silencing in different plant cells and tissues (Sarkies and Miska, 2014). In addition, sncRNAs have been found to play a critical role in regulating DNA methylation, histone modifications and gene silencing, thus controlling transcriptional system in the living organisms (Holoch and Moazed, 2015). During the past decades, plant miRNAs have been intensively studied. Although they have a small molecular size (21-24 nt), miRNAs roles are so important in the regulation of various biological processes by leading aim mRNA repression, either through degradation or translation inhibition (Wu, 2013). To play its function ideally, miRNAs has complementary sequences closely matching their respective

mRNAs targets. The binding of miRNAs to their complementary sequences confer them the feature of gene expression regulation, and this illustrates an improved image of plants miRNA targets compared with their counterparts in animals, where in the latest there are only a few regions of miRNA which restrict the complementary action (Xu et al., 2017). However, there is biochemical and genetic indication that through repressing and translation, many miRNAs will regulate their own targets (Bordersen et al., 2008; Prathiba et al., 2017). MiRNAs have key functions in controlling the plant differentiation, transition from the vegetative to the reproductive phase, morphogenesis of reproductive organs, phytohormones stimulation, stress response regulation and they may even control the pathway of their own biogenesis (Zhao et al., 2017).

There is an interesting miRNA property, that it can move from one cell to another (cell-to-cell movement), probably via plasmodesmata. Such a property allows miRNA to play the regulatory role in the differentiation of various cells and organs where they have been synthesized (Kitagawa and Jackson, 2017).

The molecular basis for the formation and maintenance of epiallelic states of many identified epialleles in many flowering plants was provided by the RNA-directed DNA methylation pathway (Law and Jacobsen, 2010). This pathway makes a feedback loop between small interfering RNAs (siRNAs) and DNA methylation that represses the gene expression and enables propagation of epiallelic states through both mitotic and meiotic divisions. In addition, the existence of siRNAs provides sequence-specific guides that simplify silencing at distant loci, even on different chromosomes (Chow and Ng, 2017).

Eventually, sncRNAs can play a pivotal role in the phenomenon of heterosis by guiding DNA methylation via the RNA-directed DNA methylation pathway. Recently, the patterns of small RNAs showed differential expression in hybrids compared with their respective parents in different crops (He et al., 2010).

2-1-3. Chromatin Remodeling

Chromatin is the most condensed and complicated form of DNA. This compacted form ensures the fitness of DNA package into the nucleus, protects the DNA structure, organizes gene expression and controlles DNA replication (Rajewsky et al., 2017). Chromatin shows two different coiling intensities, euchromatin, which is the less coiled and compacted shape, and the heterochromatin, which has opposite properties as being more coiled and compacted (Santos et al., 2017).

During the plant cell cycle (mitosis and meiosis), chromatin pass-through various dynamic structural changes termed "Chromatin Remodeling". These structural changes serve in mediating the attachment between transcription factors and their respective DNA sequence targets (Arya et al., 2010).

In many occasions, chromatin remodeling exposes its action by facilitating the passage of transcriptional factors to the nucleosome octamer core, this will eventually permit aberrant pattern of gene expression (Secco et al., 2017).

The modulation of chromatin depends on the disruption of the nucleosome macromolecules-DNA package which represents the basic structural unit of DNA (Goldstein et al., 2013). Several findings clearly demonstrated that the epigenetic changes of chromatin remodeling could be transmitted via successive generations, and this mode of action acts like a cell memory that provokes organism to acclimatize and overcome stress conditions (Lämke and Bäurle 2017; Santos et al., 2017).

RNA and DNA polymerases are key players in the chromatin re-modulation process with aid of SWI2/SNF2 proteins family. The two polymerases form a complex of SWI2/SNF2 and two copies from each, H3 and H4 dimer histones. The incorporation process requires energy in the form of ATP (Clapier et al., 2017).

The required energy will guarantee smooth access of the transcriptional factors to the DNA nucleosome. Although the DNA still wrapped around the histone octamer, this process will make the chromatin less compact and more flexible in a norm of action similar to acetylation (Rajewsky et al., 2017).

The SWI2/SNF2 has been demonstrated by various regulation functions in the biosystem. Based on its function and phylogenetic pathway, the SWI2/SNF2 family could be grouped into several subfamilies. The most important member is BRAHMA (BRM) which plays a pivotal role in a number of vital biological events especially post-embryonic stages via regulation of gene activation and suppression (Zhang et al., 2017). Furthermore, the importance of BRM is widening it may activate the upstream regulation of the transition to the reproductive phase (Yang et al., 2015).

2-1-4. DNA Methylation

From a biochemical point of view, DNA methylation is a chemical change which includes a covalent bonding between methyl group (CH3) and the cytosine residue in the DNA helix. This chemical modification is the most studied example of epigenetic variations and can inheritably control the dynamics of chromatin structure (Alvarez-Venegas et al., 2014), through what became known as "Cell Memory". Thus, it plays a vital role in various biological activities, for instance activation and silencing of transposable elements and the regulation of gene expression (Köhler and Springer, 2017).

Developmental abnormalities may result from alterations in DNA methylation, and such defects can be induced by the classical genetic mutations. In other words, it is like a combination of sudden genetic modification accumulated in backgrounds of hypomethylated DNA resulting in heritable epigenetic mutations "Epimutations" (Fortes and Gallusci, 2017).

Variation in DNA methylation is so available between individuals that belong to wild or domestic populations. Therefore, it is suggested that the plant phenotypic variation caused by epialleles may be wider than what has been foreseen. In fact, the level of cytosine methylation is highly different from one taxon to another in the plant kingdom and it may reach 30% of the total cytosine. Also, it has been found that there is a higher level of DNA methylation in monocots compared with dicots (Rajewsky et al., 2017).

In plants, DNA methylation happens at cytosine residue in different sequences, asymmetric CHH and both CHG and CG symmetric status (H is any nucleotide except G). Through meiosis and mitosis, patterns of DNA methylation are propagated through different pathways by distinct DNA methyltransferase enzymes (Catania et al., 2017). Methyltransferase 1 and the unique plant chromomethyl transferase maintain the CHG and CG methylation via DNA replication, whereas methyltransferase 2 establishes DNA methylation in all sequence status by a small RNA-directed DNA methylation pathway (Law and Jacobsen, 2010). There is a great similarity between DNA mutations and heritable DNA methylation, as both can take place in a state of induced or natural manner. The creation of novel epialleles will affect the gene expression that in turn will lead to aberrant final products, thus a de novo phenotypic traits will emerge (Becker and Weigel, 2012; Schmitz and Ecker, 2012; Rajewsky et al., 2017).

The genetic architecture of many individuals is the main cause of most variations in DNA methylation that were observed among them. For example, the polymorphism among individuals, such as repeated sequences or transposons, will determine the epigenetic status of epialleles which are haplotype specific (Eichten et al., 2013).

Different studies focused on a comparative global genome analysis of DNA methylation in plants with limited genetic diversity (Rodrigues and Zilberman, 2015). These studies concluded that the epigenetic differentiation of individuals

was caused by spontaneous variation of DNA methylation within a little time. In many examples of natural variation of cytosine methylation display alteration in the cytosine methylation level from time to time, which indicates the potential of semi stable heritability of epigenetic information (Gutzat and Scheid, 2012).
The outcomes of another study which was performed on some inbred lines and their hybrids were different (Lauria et al., 2014). The used MSAP analysis indicated that the variation of DNA methylation was due to alterations in CHG or CG/CHG methylation at the same time. The authors added that novel methylation of the unmethylated alleles revealed all changes of DNA methylation, and 88% of these changes in DNA methylation were inherited.

2-2. The MSAP procedure

The MSAP procedure is widely used technique in the biotechnology for molecular cloning, genetic mapping and identifying the variations in the DNA methylation levels within the individuals and among them in different species including maize (Meng et al. 2012, Shan et al. 2013 and Yu et al. 2013). Reyna-Lopez, et al. (1997) was the first proposed this modern technology which is in fact a modified AFLP (amplified fragment length polymorphism) protocol. Typically, the MSAP technique banks on bisulfite conversion, restriction enzymes to digest the double stranded DNA as well as ligating adaptors to link the primers with the sticky ends of the fragments. The MSAP procedure includes two methylation-sensitive restriction enzymes, *Msp*I and *Hpa*II isoschizomers recognize the same tetranucleotide sequence (CCGG) but with differential sensitivity to methylation of cytosine. In addition to *Eco*RI enzyme distinguishes hexanucleotide sequence (GAATTC) to create four sticky ended nucleotides with 5'end (AATT). *Msp*I can separate the unmethylated plus hemimethylated or fully methylated regions, while *Hpa*II can digest the unmethylated and hemimethylated recognition sites

(Schulz, et al., 2013). Naturally, the restriction process is a defensive mean that followed by prokaryotic and eukaryotic cells for their protection from intruder nucleic acids with no damage to their ones by methyltransferase. Type I restriction enzymes (including *Eco*RI *and Msp*I) are multifunctional enzymes those capable of both modification and restriction activities based on the DNA methylation status. This type of enzymes requires ATP as a co-factor in contrary with type II enzymes which their co-factor is the magnesium ions (Mg^{2+}). Basically, type II restriction enzymes cleave the phosphodiester bonds of double stranded DNA to result palindromic and undivided homodimers sequence 4-8 nt in length (Geoffrey et al., 2012).

2-3. The Role of Methylation in Hybrid vigor

Hybridization is the most important tool that maintained a strong incidence in the traditional breeding programs in spite of the tremendous evolution of molecular markers (Goulet et al., 2017).

In the long run of evolution, plant breeders can guide the local adaption through the transgressive segregation and creation of novel alleles and/or epiallels, which in turn will result in the formation of new hybrid species (Bradshaw, 2017). The three proposed models; dominant, over-dominant and epistasis have achieved remarkable success, however, they did not award distinct understanding of heterosis because each has weakness points (Khotyleva et al., 2017). Moreover, these models cannot take all aspects of the heterosis and in different occasions they failed to give full explanation of the phenomenon (Groszmann et al., 2013). Plus genetic effects, heterosis could be affected by the category named "epigenetic" effects, which represents a part of non-Mendelian inheritance, cell fate and regulation of gene expression (McKeown and Spillane, 2014).

Different modifications can motivate epigenetic effects consequently the same genotype may result in different phenotypes (Alvarez-Venegas et al., 2014). It has been proven through different studies that the epigenetic effects of cytosine methylation can contribute to the development of heterosis phenomenon. Such studies have exposed differences in cytosine methylation patterns in heterotic F1 hybrids in maize compared with their respective parents (Zhao et al., 2007), (Figure, 1).

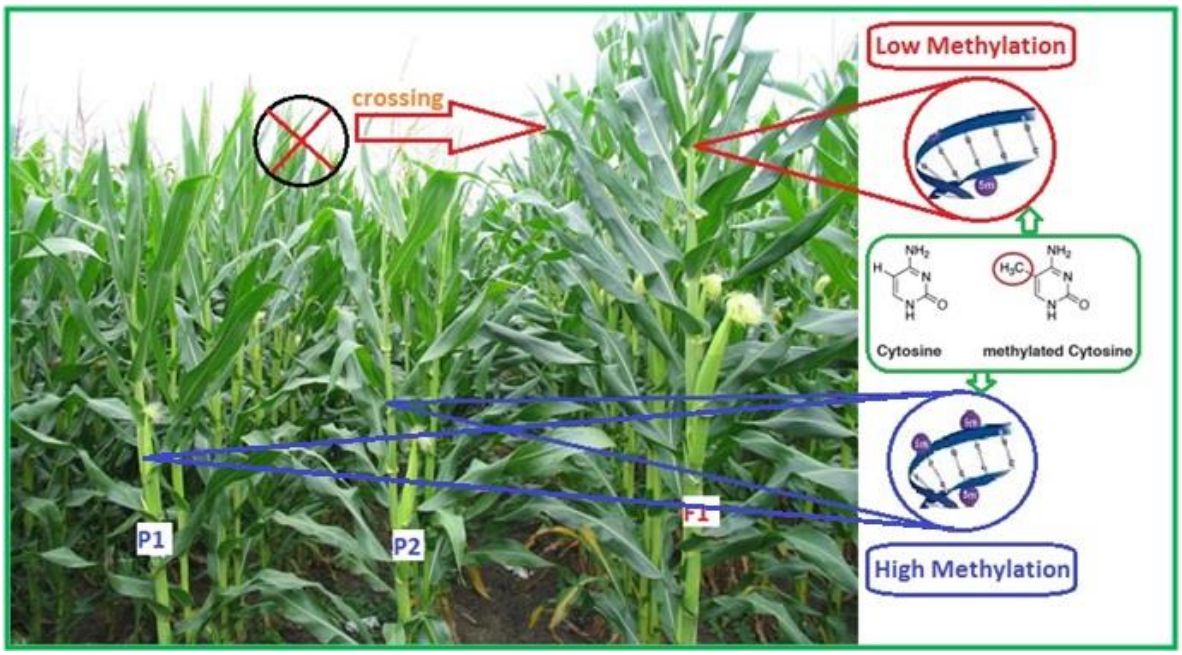


Figure 1: The proposed effect of hybridization in altering DNA methylation.

A molecular analysis of the distributed methyl groups in the genomes of 11 maize inbred lines and their respective half diallel hybrids has been conducted (Yang et al, 2011). The analyzed data of methylation sensitive amplified polymorphism (MSAP) technique revealed a negative relationship between methylation level and hybrid vigor for the number of kernel rows trait, but the relationship goes in the opposite direction as being positive for kernels no. trait. However, the trait of 300 kernel weight did not correlate with the methylation level in hybrids genome.

The same technique was followed by Eichten et al. (2013) when they studied the Differentially Methylated Regions (DMRs) in the genomes of 20 maize parental lines. They succeeded in detecting 1966 common DMRs and 1754 rare domains. Most of the detected DMRs were inherited, and nearly half of the total DMRs were found to be highly related with single nucleotide polymorphism within DMRs domains or next to it. There was not only a high significant relationship between DNA methylation and gene expression pattern, but the latest was affected by the DMRs even in relatively distant regions.

The epigenetic variations may represent a new genetically independent source for phenotypic variations (Lauria et al., 2014). The researchers tried to track the variation in the DNA methylation pattern across eight generations of maize inbred lines by using MSAP. The authors came up with a really interesting conclusion that 12% of DNA methylation has been memorized by the maize plants genome and transmitted through six generations. The cytosine residue was differentially methylated from one individual to another at a ratio of 7.4%.

Two hybrids with their inbred parents have been selected to monitor the possible alterations in the DNA methylation level at different organs and growing stages (Liu et al., 2014). The DNA methylation was analyzed in embryos and endosperm after 15 days from fertilization, and in leaves and primary roots at germination stage. The methylation level in all hybrids organs was less than their counterparts the inbreds, and de-methylation was at its highest level in the hybrids which indicates its tendency to permit higher levels of gene expression and de-activation of gene suppressor. The total of polymorphic DNA fragments reached 63 fragments, 11 out of these were found to encode well known functional proteins in maize.

An understandable relationship was detected between the level of DNA methylation and the phenotypic performance of eight maize parental lines and their half diallel hybrids (Almelhami, 2017). The results pointed to significant

alterations in the DNA methylation pattern due to hybridization, and this has a key role in hybrid vigor showed by the derived half diallel hybrids. Estimation of DMRs (Differentially Methylated Regions) revealed a general decline in the DNA methylation level in the hybrids population compared with their parental one. The percentages of unmethylated and hemimethylated regions were 13.1% and 9.9% in hybrids while they were 9.5% and 6.6% in parents, respectively. The percentage of internal cytosine methylation in the parental population was 20.7% compared with the same percentage in the hybrids population (19.5%). Also, parents showed a higher percentage of full methylation or absence of target (63.2%) against this percentage in their half diallel hybrids which was a bit low (57.6%).

2-4. The Role of Inbreeding in DNA Methylation

Previous studies of heterosis have revealed feasible mechanisms to estimate the better vigor, but the mechanisms that lie beneath this multifaceted phenotype are still poorly understood, and the role of DNA methylation in heterosis is indistinguishable (Chodavarapu et al., 2012).

The understanding of heterosis mechanism is based on the accurate sympathetic of inbreeding depression. Classically, the definition of inbreeding depression includes two parts, the first is the mating between two individuals being identical in their genetic composition which represents the cause and will be resulted in the second part which is the depression of the offspring general performance. Usually, in cross-pollinated crops, self-pollination leads to the production of inbred lines to be a keystone in the production of hybrids through crossing process (Shuro, 2017). Despite the obvious effect of repeated self-pollination and its combination of deleterious alleles, the adoption of this idea did not help so much to set a convincing explanation of the hybrid vigor shown by genotypes propagated by self-pollination (Hartfield et al., 2017).

In the light of modern epigenetic facts, there are still many questions that need to be answered. For instance, does the inbreeding process lead to gathering harmful "epialleles" as it gathers harmful alleles. Epialleles are genetically identical alleles but at the same time vary in the way they are epigenetically modified (Lauss, 2017). Inbreeding process is accompanied by many genetic as well as epigenetic variations including DNA methylation, which is considered the most prominent epigenetic change. Many studies proved that the variation in DNA methylation between inbred parents and F1 hybrids indicating the importance of inbreeding in the development of epigenetic variations (Vergeer et al., 2012).

Song et al., (2010) suggested that the majority of gene-expression changes in hybrids are not associated with cis-acting DNA methylation changes, and instead indicate that trans effects may mediate the majority of the transcriptional differences in hybrid offspring. They added that it is possible that a subset of the gene-expression changes may also be caused by intergenerational epimutations in the hybrids.

Other efforts have focused on the role of DNA methylation in updating the "epigenetic memory" either during the plant development (mitotically) or among individuals belonging to the same species but at different genetic backgrounds (meiotically), where the patterns of DNA methylation can vary (Eichten et al., 2011). The study was performed on maize inbred lines which were developed by at least four generations of self-pollination followed by three back- crosses. The analysis of DMRs reflects the relatively stable inheritance of the DNA methylation pattern.

A recent study reported that the cytosine methylation increasingly occurs in the hybrids compared with their parents (Shen et al., 2012). This proved the impact of hybridization in the modification of DNA methylation level.

To evaluate the long-term inheritance of DMFs, two plants from the second generation were advanced to the sixth generation by self-pollination (Lauria et

al., 2014). Authors identified 15 out of 102 methylated regions to be DMFs in different members of the first generation. Part of the original DMFs has been transmitted to the next generation in a ratio ranging from 50 to 72.5%. MSAP analysis assured that 82% (14 out of 17) of the studied DMFs were still measurable in the sixth progeny. These results indicated that at least 12% (11 out of 87 at leaf 2 stage; and 11 out of 93 at leaf 9) of the total DMFs originally identified in the base population generation were meiotically inherited for six generations.

The detected phenotypic differences among inbred lines can be affected by epigenetic variation including methylation variation which was found to be highly related to gene expression (Feng et al., 2015). Generally, authors could not link between the heterozygosity of the DMRs and the levels of DNA methylation. Meanwhile, they reported that there is evidence about 85% of the assays showed expected level of DNA methylation, whereas just 5 out of 150 assays revealed exclusive shifting in the methylation status and other (17 out of 150) of assays showed a partial increasing or decreasing in DNA methylation.

2-5. Quantitative Genetic

2-5-1. Diallel Cross

Selection of inbred parents is crucial in the application of hybrid breeding programs (Katba et al., 2017). Thus, the suitable matting system is a prerequisite that enables researchers to identify the genetic behavior of parental lines (Birhanie et al., 2017). Selective parents can be used to grant desirable characteristics and use them in the production of high yielding hybrids (Raut et al., 2017).

Successful reproduction and outcrossing are affected by strategies of the matting system which is extraordinarily varying among plant species (Darwin, 1876). Effect of these strategies appeared distinct on the demographic distribution and genetic structure of the population (Goldberg et al., 2010).

From the most effective mating systems in the identification of the organism genetic structure underlying both quantitative and qualitative traits is the diallel crossing design. This crossing scheme is widely practiced by geneticists and plant breeders for its effectiveness in the assessment of general and specific combining ability of different genotypes (Zhang et al., 2015).

2-5-2. Hybrid Vigor

Agricultural sector faces big challenges to cope with the large growth in both human and animal populations. The great versatility of the new uses due to rapid innovative technology has placed additional pressure on the field crops productivity (Postnote, 2017). In the recent years, key crops have been rapidly developed via new efficient breeding tools, in which plant breeders were able to introduce novel or modify available traits. Regardless of the multiplicity of plant breeding techniques, heterosis was the most important stanchion in the green revolution of the 1960's (Li et al., 2017).

To develop inbred lines, plant breeders must possess enough information about the genetic diversity and heterotic groups that enable them to utilize germplasms more efficiently and increase the outcomes of the hybrids breeding program (Meena et al., 2017).

We have discussed earlier that the real reasons underlying heterosis have not been resolved till now, since most of the hypotheses that have been launched so far succeeded in explaining a certain aspect of the phenomenon in a certain circumstance. For decades, many scenarios have been developed to explain heterosis, although none of them have been definitive. Hence, the phenomenon appears to be complex in terms of the complexity of both genetic and epigenetic aspects of the living organism genome (Herbst et al., 2017).

Although, heterosis is debated for a long time, ironically, even molecular means did not go far beyond what had been achieved under traditional genetics. What makes it even more complicated is that the molecular reasons behind the hybrid vigor are being challenged by the segregation of the F1 generation, which makes it non-traceable (Ryder et al., 2014).

The used expressions to describe the hybrid vigor are somewhat different, but they rotate in the same ark as the phenomenon occurs when the offspring expose values of growth, yield, adaptation and/or any other trait beyond their parents. Remarkably, heterosis is a bidirectional phenomenon as it can be either towards the increment or the decrement in the trait mean, compared with the best or the mid parents mean (Marcon, et al., 2017).

Historically, the first written notes about heterosis were introduced by the botanist Joseph Kolreuter around 1766, as he compared between the early and late hybrids of tobacco. He stated that the early hybrids tended to intermediate its inbred parents being more plentiful, whereas, the late hybrids tended to act similar to their parents (Goulet et al., 2017).

Through performing self-pollination, Darwin (1867) characterized heterosis in inbred lines and then crossed parental pairs of sixty plants species. He noticed

that the F1 progenies expose transgressive performance in height and vigor exceed their inbred parents. Although heterosis term often associated with genetic diversity which is largely true, it cannot be completely generalized. Genetic variability has been a target for early farmers and plant breeders since the earliest attempts to cultivate plant species, and without it, there would be fewer opportunities for plant improvement in the desired direction. There is a widespread desire to develop new molecular means to interpret and predict the hybrid vigor (Bhandari et al., 2017).

After all the controversy raised about the genetic basis of hybrid vigor in its both traditional and molecular aspect, the term "Epigenome" emerged strongly as one of the reasons leading to the deviation of the offspring performance (Lauss, 2017).

The great diversity of molecular genetic and epigenetic markers gave it the advantage of the possibility of use for various purposes and this did not interfere with its highly environmental independence (Muhammad et al., 2017).

Five maize inbred lines were fully mated to estimate heterosis and some other genetic parameters (Al-Falahy, 2015). Most of crosses revealed significant estimates of heterosis ranged between positive and negative values for tasselling (-3.4 to 4.7%), silking (-3.3 to 4.5%), leaf area (-73.2 to 216.8%), number of rows per ear (-2.4 to 1.0%), number of kernels per row (-1.3 to 11.8%), 300 kernel weight (-19.0 to 15.7%) and grain yield (-41.7 to 67.1%). Plant and ear height relied on different gene action as single hybrids showed only significant positive values of heterosis for these traits (31.1 to 236.4%) and (2.25 to 28.5%), respectively.

At the aim of estimating heterosis, Abdul-Hamed et al. (2017) performed a study followed Line x Tester analysis on seven maize lines to generate twenty hybrids. Most of crosses revealed positive or negative significant heterosis against their best parents, which ranged between 1.47 to -8.88%, 38.08 to -22.58%, 7.09 to -

12.6%, 15.07 to -21.93% and 42.46 and to -10.53% for silking, leaf number, rows per ear, 1000 grains weight and grain yield per plant, respectively.

According to a North Carolina Design II mating scheme, ninety six crosses were generated from mating between twenty four maize lines in order to estimate combining ability and heterosis. All crosses revealed significant heterosis for all studied traits. Positive heterosis was a portion of plant height and grain yield reached 60.8% and to 322%, respectively. The negative significant heterosis was restricted on silking ranged and its highest value was -12.7% (Adebayo et al., 2017).

Ali et al., (2017) adopted full mating design among six maize inbred lines. The authors mentioned that trait means differed significantly, and accordingly heterosis ranged from -10.8 to 0.6% for anthesis, -9.7 to 0.6% for silking, -8.1 to 15.5% for plant height, -11.5 to 16.0% for ear number per plant, -7.4 to 9.1% for grain rows per ear, -8.9 to 18.5% for grain number per row, -3.9 to 19.5% for 1000 grains weight, and -16.1 to 51.3% for grain yield.

A study was performed on ten lines with their single crosses and four checks. The analysis of variance revealed significant differences in all studied traits (anthesis, silking, grain rows per ear, grain weight and grain yield). Most of the hybrids exhibited positive significant hybrid vigor percentages for yield and yield contributing traits. The highest positive heterosis reached 15% for grain rows per ear and 42% for plant grain yield. Several hybrids showed negative significant heterosis ranged from -34% for anthesis and silking to -28% for maturity. Only two hybrids owned significant vigor reached 6.70% and 5.59% for 100-grain weight (Bisen et al., 2017).

Similar findings were stated by Li et al., (2017) as they used eleven maize inbred lines with their single crosses to assess heterosis. The maximum heterosis values reached -4.4%, -5.0%, 27.9%, 36.5%, 7.6%, 3.1%, 7.2% and 91.4% for anthesis, silking, plant height, ear height, leaf number above ear, leaf number below ear, kernel rows and grain yield per plant, respectively.

Heterosis was estimated in the single hybrids descended from different diallel combinations among seven maize inbred lines. The over dominance gene action prevailed the inheritance of most of the studied traits as majority of F1 progeny showed positive heterosis percentages (1.48 to 11.62%), (1.06 to 12.02%), (0.51 to 26.33%), (3.78 to 39.33%) and (0.68 to 2.72%) for anthesis, silking, plant and ear height and physiological maturity, respectively, whereas positive and negative heterosis recorded for 1000-grain weight and plant yield (-21.72 to 11.73% and -56.59 to 15.60%), respectively (Matin et al., 2017).

2-5-3. Relationship between Inbreeding and Hybrid Vigor

Plant performance is affected by the high frequency of allele homozygosity, consequently, plant productivity and vigor will decrease due to inbreeding depression (Mulualem and Abate, 2016).

Inbreeding which means the mating between closely related individuals having identical genetic architecture and descending from a common ancestor, will cause a sharp decline in the general fitness due to decreasing of useful heterozygosity at specific loci and accumulation of recessive deleterious alleles (Pemberton et al., 2017). The phenomenon which is contradicting the phenomenon of heterosis became a corner stone in the breeding programs of cross-pollinated species (Paige, 2010). To develop hybrids and synthetics maize, creating parental inbred lines is fundamental. Therefore, genetic diversity and the high level of homozygosity are the two main pillars for developing new maize hybrids (Pabendon et al., 2008).

Conventionally, inbreeding depression is one of the early studied biological phenomena in an extensive way. Unfortunately, it has not received the same attention at the molecular level hence it is still poorly understood (Kardos et al., 2016). Perfect parental inbred pairs which expected to expose higher hybrid vigor are not necessary to be with the maximum level of genetic distance,

nevertheless, it may be derived from parental inbred pairs with a low value of genetic distance hence this could be easily detected among individuals belonging to the same population (Pabendon et al., 2010). In many occasions, genetic purity and the level of homozygosity of an inbred line could be detected through the uniformity of its phenotypic traits (Kawamura et al., 2016). However, selection of genetic diversity and homozygosity level based on yield and other morphological traits will be less accurate. Therefore, the more precise evaluation could be obtained via molecular markers in general and epigenetic markers in particular (Sorkheh et al., 2017).

Using 36 white maize inbred lines, Abakemal et al. (2014) tested the effect of selection on the nutritional value of QPM lines. Selfing was conducted for four generations, the majority of inbred lines revealed higher values of heterozygosity than expected, ranged 8% to 16.7%. Such findings encouraged authors to implement additional self-pollination for more purity as a considerable ratio of heterozygosity still retained (6.25%). Using these inbred lines in the hybrid program is not effective with reliance only on self-pollination and then cross-pollination between pair because the level of heterozygosity will be high (Efendi et al., 2015). A study was performed by the previously mentioned authors using various populations of inbred lines, their results showed that the homozygosity level has differed significantly in the advanced levels (beyond the 6^{th} generation). The derived data from the previous study indicated that just one of inbred sets was with homozygosity level exceeded 85%, whereas the heterozygosity levels ranged between 2.6 to 40% in the rest of inbred sets.

2-5-4. Combining Ability

Hybrids development necessitates heterotic populations and effective programs to improve versatile combiners as fresh start in any breeding program. The identification of heterotic groups of parental inbred lines and their combining abilities will facilitate successful hybrids production through the selection of breeding strategies and inbred parents (Meena et al., 2017). Therefore, every study has been performed to predict the overall performance of the hybrids and their inbreds parents should initially estimate the effect of combining ability and the gene action between genotypes (Liton et al., 2017).

Sprague and Tatum (1942) were the first who stated that the effect of GCA is correlated with the effect of additive gene action, whereas SCA effect is correlated with non-additive gene action. This information gives a previous distinct indication about the genetic nature of genotypes which enable the breeders to select genotypes with desirable traits and determine the kind of relationship among them (Ai-Zhi et al., 2012).

Five maize inbred lines were crossed in a full diallel mating design to estimate heterosis and combining ability (Al-Falahy, 2015). Effects of both general (GCA) and specific (SCA) combining ability were found to be significant for most of studied traits (tasseling, silking, plant and ear height, leaf area, 300-kernel weight, number of kernel rows, number of kernels per row and grain yield), which indicated the importance of both kinds of gene action, additive and non-additive in controlling the estimated traits. The highest SCA effects ranged between its positive (9.34) and negative values (-9.91) for yield components.

The additive gene action exhibited full control over growth and yield traits (anthesis-silking interval, number of kernel rows per ear and number of kernels per row) in a study conducted on 27 inbred lines with four testers during three seasons (Anilkumar et al., 2017). The authors' conclusion was based on GCA/SCA ratio which was greater than unity.

Similar findings were detected by Aslam et al. (2017) as they performed a diallel cross among six maize inbred lines. The estimated effects of general and specific combining abilities were in different significant ranges for grain rows per ear (-1.62 to 0.87) and (-0.27 to 0.65), Grains per row (-0.38 to 2.75) and (-0.95 to 3.19) and for 100 grain weight (-2.55 to 2.83) and (-2.59 to 2.29), respectively. Thus, GCA/SCA ratio which was greater than unity for the quantitative traits except for grains rows per ear trait revealed greater importance for the additive gene effect in the inheritance of these traits. The combined analysis of variance for anthesis, silking, anthesis-silking interval, plant height, grains yield and 100-grains weight revealed highly significant differences among genotypes (Bawa et al., 2017). Both GCA and SCA effects were highly significant for all traits. The ratio of GCA/SCA indicated the prevalence of the additive gene action in controlling the inheritance of these traits as being greater than unity for all studied traits except for anthesis, which exposed non-additive gene action.

Dar et al. (2017) estimated the general and the specific combining abilities for yield and yield correlated traits (tasselling, silking, plant height, ear height, grain rows per ear, grains per row, 100-grain weight and grain yield). Although, both GCA and SCA effects were significant for previously stated traits, authors concluded that genes with additive effect were more valuable in the inheritance of these traits.

For the aim of determining the combining ability and the heterotic groups, seventeen maize inbred lines were crossed in a diallel scheme by Konate et al., (2017). The additive (GCA) and non-additive (SCA) gene action were significant for grain yield and most of the other traits such as silking, anthesis, plant height, ear height and ears per plant. The GCA variance was the greater than that belonging to SCA, which indicated the superiority of the additive gene in revealing these traits.

The genetic analysis of mean squares showed a high significance of both GCA and SCA effects for silking (17.2 and 11.7), plant height (1119.4 and 1070.4), ear height (368.0 and 283.1), respectively. The non-additive gene action was not significant for 1000-grain weight, while the GCA effects were significant and their maximum value was reached 3313.9. Plant yield was on the opposite attitude with significant effects restricted on SCA (4.29), (Matin et al., 2017).

The combining ability in general and specific mean was significant for all studied traits (anthesis, silking, plant height, ear height, maturity, kernel number per plant, grain row per kernel, grain number per row, 100-grain weight and grain yield per plant), (Wani et al., 2017). GCA of the ten inbred lines ranged from negative to positive significant estimates (-0.81 to 0.80), (-1.14 to 0.64), (-8.91 to 7.33), (-5.50 to 3.82), (-1.51 to 1.79), (-0.07 to 0.05), (-0.20 to 0.42), (-0.65 to 0.64), (-0.81 to 0.69) and (-3.67 to 7.37). The forty five crosses revealed SCA effects in nearly the same ranges (-2.43 to 4.04), (-3.06 to 4.41), (-60.13 to 72.55), (-12.69 to 26.95), (-7.06 to 7.33), (-0.22 to 0.32) , -(0.64 to 1.95), (-1.73 to 6.36), (-1.16 to 4.85) and (-6.10 to 65.49) respectively. As GCA/SCA ratio was found to be greater than unity for anthesis, silking and maturity traits this cleared greater importance for the additive gene action in revealing these traits.

Both additive and non-additive effects showed their crucial role in the inheritance of the studied traits (silking, plant height and grain yield) in a study performed on 26 maize inbred lines with their single crosses and two checks (Wolde et al., 2017). In view of the significant effects of GCA and SCA combining abilities, which ranged between positive and negative values, the genes of additive and non-additive action effectively controlled the mentioned traits.

Chapter Three

3. Materials & Methods

3-1. Quantitative Genetic Study

3-1-1. Field Trails

A field trial was conducted at the Field Trails Station in the Department of Field Crop Sciences / College of Agriculture / University of Anbar in the alternative site (Abo Ghraib- Baghdad) for two growing seasons, spring and fall of 2016.

3-1-1-1. Spring Season 2016

Twenty original and self-pollinated progenies of inbred lines of maize were received from the supervisor Assis.Prof. Dr. Ayoob Obaid Alfalahi, and at the end of the growing season the best five inbred lines were selected.

All the necessary processes for land preparation were established. Post tillage, the recommended dosage of super phosphate fertilizer (P_2O_5) was incorporated in the soil at a rate of 400 kg ha^{-1}. While the recommended dosage of nitrogen fertilizer (400 kg ha^{-1}) was applied in the form of urea (46% N) in two portions, 200 kg ha^{-1} of each. The first one was applied prior planting, whereas, the second was applied when seedlings height reached 0.4-0.5 m. The field was divided into ridges, each of 10 m long and 1 m apart to facilitate the movement and the crossing process, as well as to provide a sufficient distance for a healthy growth. Seeds of both, base and self-pollinated ears of the twenty inbreds were planted in 15th of March 2016 in a way of ear to row (ETR) in this growing season. Holes were overplanted then thinned to one seedling per hole.

The experiments were conducted under irrigated conditions (irrigation was conducted as needed) and the field was kept free of weeds with the aid of Atrazine herbicide (4.5 l ha^{-1}). Corn borer (*Sesamia cretica*) was controlled in both seasons at seedling stage of 6 leaves by using liquid diazenon (1.4 ml l^{-1}).

According to vegetative growth vigorous, top 5 self-pollinated inbred lines were selected to accomplish half diallel mating, alongside with their counterparts original population. Before silk protrudes, the emerged ears were covered by paper bags, to ensure guided pollination. As tassels started shedding pollens, they were covered with bigger paper bags. In the early morning of the next day, the pollens were collected to do the necessary half diallel crosses within both, base and self-pollinated populations. All inbred populations were propagated by sibbing. Crossed ears were re-covered till its maturity, then they were harvested and left to dry then detached individually.

3-1-1-2. Fall Season 2016

The comparison trial was conducted in this season, where, both populations (30 genotypes), original population (5 parental inbreds and their 10 half diallel hybrids) and self-pollinated population (5 parental inbreds and their 10 half diallel hybrids) were planted in 29th of July in ridges (0.25 x 0.75m), four ridges for each genotype with 4 m long.

Holes were overplanted and thinned later to one seedling per hole. The genotypes were randomly distributed according to RCBD design with three replications. Land preparation and crop management were performed as previously stated in the previous growing season. Ten plants from each genotype were randomly chosen to record the data for the necessary traits of each genotype.

3-1-2. Phenotypic Traits

1. Days to 50% anthesis (Day): The mean of days number from planting date to 50% anthesis.
2. Days to 50% silking (Day): The mean of days number from planting date to 50% silking.

3. Plant height (cm): The mean of 10 plants height measured from the ground level to the flag leaf node.
4. Ear height (cm): The mean of 10 plants ear height measured from the ground level to the main ear node.
5. Leaves number (Leaf plant^{-1}): The mean of functional leaves number of 10 plants from each treatment.
6. Leaf area (cm^2): The mean of leaf area by measuring the length and the maximum width of main ear leaf of 10 plants in each treatment with the aid of the following formula:

   Leaf area (cm^2) = leaf length (cm) × leaf width (cm) × 0.743 (Stewart and Dwyer, 1999).
7. Tassel length (cm): The mean of tassel length of 10 plants measured from the base of the tassel lowest branch to the tassel tip.
8. Tassel branches number (Branch tassel^{-1}): The mean of the main branch number in 10 plants tassels.
9. Ears number (Ear plant^{-1}): The mean of ears number of 10 plants in each treatment.
10. Kernels rows number (Kernel rows ear^{-1}): The mean of kernels rows numbers of 10 ears in each treatment.
11. Kernels number (Kernel row^{-1}): The mean of kernels per row of 10 ears in each treatment.
12. Kernel weight (gm): The weight of 500 kernels in each treatment.
13. Grain yield (gm): The total grain yield of the 10 harvested plants divided by 10.

3-1-3. Statistical and Genetic Analysis of Phenotypic Traits

According to the data of individual plants, analysis of variance was conducted to reveal the significant differences between treatments following RCBD design. The significance level was 5% as least significant difference test has been used to compare between genotypes performance based on trait means.

- The following linear model of RCBD design was used:

Yij $= \mu + ti + bj + eij$

Where:

Yij: the observation value of i treatment in block j.

μ *: grand mean of the trait.*

ti : the effect of i treatment.

bj : the effect of block j.

eij: the value of experimental error of i treatment in block j.

- **Hybrid vigor for each cross was estimated as the percentage of F1 over the best parent (Laosuwan and Atkins, 1977).**

Heterobeltiosis (H%)=[(F1 - BP)/BP] 100.

Where:

F1 = the performance of the hybrid

BP = the performance of best parent.

- **Analysis of Combining Ability**

Based on the result of *F*-test, the traits with significant differences were analyzed for combining ability effects following the second approach of Griffing (1956) diallel analysis with the First model (Fixed). The linear model was as follows:

$$Y_{ijk} = \mu + g_i + g_j + S_{ij} + R_k + e_{ijk}$$

Where:

Y_{ijk}*: the observation value of experimental unit (for ij genotype in block k.*

μ *: grand mean of the trait (grand effect).*

g_i*: the effect of general combining ability of i genotype.*

g_j*: the effect of general combining ability of j genotype.*

S_{ij}*: the effect of specific combining ability of ij hybrid*.

R_k*: the effect of block k.*

e_{ijk}*: the effect of experimental error.*

Table 1: Analysis of variance of diallel mating design according to Griffing (1956) Second Approach-Model I (Fixed).

S.O.V	DF	Sum of square (SS)	Mean Square (MS)	E.M.S.
Replicates	r-1	$\frac{\sum Y_{..k}^2}{P(P+1)/2} - \frac{Y_{...}^2}{rP(P+1)/2}$	$\frac{SSr}{r-1}$	$\sigma_e^2 + \frac{P(P+1)/2}{r-1}\sum Y_{..k}^2$
Genotypes	$\frac{P(P+1)}{2}-1$	$\frac{\sum Y_{ij.}^2}{r} - \frac{Y_{...}^2}{rP(P+1)/2}$	$\frac{SSgen}{[P(P+1)/2]-1}$	$\sigma_e^2 + \frac{r(P-2)}{P-1}\sum g_i^2$
GCA	P-1	$\frac{1}{(P+2)}(\sum Y_{i.} + Y_{ii})^2 - \frac{4Y_{...}^2}{P}$	$\frac{SSgca}{P-1}$	$\sigma_e^2 + \frac{P+2}{P-1}\sum g_i^2$
SCA	$\frac{P(P-1)}{2}$	$\sum\sum Y_{ij}^2 - \frac{1}{(P+2)}\sum(Y_{i.}+Y_{ii})^2 + \frac{2}{r(P+1)(P+2)}$	$\frac{SSsca}{P(P-1)/2}$	$\sigma_e^2 + \frac{2}{P(P-1)}\sum\sum S_{ij}^2$
Error	$(r-1)\left(\frac{P(P+1)}{2}-1\right)$	$SS_{total} - SS_r - SS_{gen}$	$\frac{SSe}{(r-1)[(P(P+1)/2)-1]}$	σ_e^2
Total	$\left[\frac{rP(P+1)}{2}-1\right]$			

The calculation of sum squares for the general and specific combining ability was according to Singh and Chaudhary (1985):

$$SS(GCA) = \frac{1}{P+2}\left[\sum (Y_{i.} + Y_{ii})^2 - \frac{4}{P} Y_{..}^2\right]$$

$$SS(SCA) = \sum\sum Y_{ij}^2 - \frac{1}{P+2} + \sum (Y_{i.} + Y_{ii})^2 + \frac{2}{(P+1)(P+2)} xY_{..}^2$$

***Where*:**

Yi. *= sum squares Yii for parent i and F1's where Yi is a common parent.*

Yij*: the mean of F1 hybrid resulting from crossing i with j.*

Yii*: selfing of i parent.*

Y..*: the grand mean.*

P*: number of inbred lines.*

- F test was adopted to test the significance effects of the general and specific combining ability. The significance of the general combining ability effects was tested as follows:

$$F\left[(P-1)(r-1)\left(\frac{P(P+1)}{P} - 1\right)\right] = \frac{MS(GCA)}{\overline{MSe}}$$

The significance of the specific combining ability effects were tested as follows:

$$F\left[\frac{P(P-1)}{2}(r-1)\left(\frac{P(P+1)}{2} - 1\right)\right] = \frac{MS(SCA)}{\overline{MSe}}$$

MSē: *experimental error*

The effect of the general combining ability for each parent (gi) and the effect of the specific combining ability for each F1 hybrid were estimated as in the following formulas, respectively:

$$\hat{g}_i = \frac{1}{P+2}\left[\sum (Y_{i.} + Y_{ii})^2 - \frac{4}{P} Y_{..}\right]$$

$$\hat{S}_{ij} = Y_{ij} - \left[\frac{1}{P+2}(Y_{i.} + Y_{ii} + Y_{.j} + Y_{jj}\right] + \frac{2}{(P+1)(P+2)} Y_{..}$$

***Where*:**

Yij*: the mean of F1 resulting from crossing i with j.*

Yii*: the mean of i parent.*

Yjj*: the mean of j parent.*

Yi.*: sum squares Yii for parent i and F1's where i is a common parent.*

Y.j*: sum squares Yii for parent j and F1's where j is a common parent.*

Y..*: sum squares of all parents and F1's, without reciprocals.*

The variance of both, the general and specific combining ability was estimated as stated by Singh and Chaudhary (1985):

$$\hat{\sigma}^2 g_i = (g_i)^2 - \left[\frac{P-1}{P(P+1)}\right] \overline{MSe}$$

$$\hat{\sigma}^2 S_i = \frac{1}{P-2} \sum \hat{S}_{ij}^2 - \left[\frac{P^2(P+2)}{(P+1)(P+2)}\right] \overline{MSe}$$

***Where*:**

$\hat{\sigma}^2 g_i$ *: the expected variance of general combining ability effect for i genotype.*

$\hat{\sigma}^2 S_i$ *: the expected variance of specific combining ability effect for i genotype***.**

*gi: the effect of general combining ability for i genotype***.**

*Sij: the effect of specific combining ability for ij genotype***.**

P: the number of parents involved in the cross.

$\overline{MSe}$*: the sum squares of justified experimental error of general and specific combining ability.*

The standard error of the difference between the effects of both combining abilities, and the standard error of any hybrid have a common parent was estimated by using the following formulas:

$$S.E(\hat{g}_i - \hat{g}_j) = \sqrt{\frac{2MSe}{P+2}}$$

$$S.E(\hat{S}_{ij} - \hat{S}_{ik}) = \sqrt{\frac{2(P+1)MSe}{P+2}}$$

- **Estimation of Heritability**

Heritability was estimated based on variance components of: General combining ability $\sigma^2 Gca$ for parents, specific $\sigma^2 sca$ combining ability for hybrids, and variance of experimental error $\sigma^2 e$ which represents the environmental variation, (Singh and Chaudhary, 1985):

$$\%h^2_{bs} = \frac{\sigma^2 G}{\sigma^2 P} = \frac{\sigma^2 A + \sigma^2 D}{\sigma^2 A + \sigma^2 D + \sigma^2 e} = \frac{2\sigma^2 gca + \sigma^2 sca}{2\sigma^2 gca + \sigma^2 sca + \sigma^2 e}$$

$$\%h^2_{ns} = \frac{\sigma^2 A}{\sigma^2 P} = \frac{\sigma^2 A}{\sigma^2 A + \sigma^2 D + \sigma^2 e} = \frac{2\sigma^2 gca}{2\sigma^2 gca + \sigma^2 sca + \sigma^2 e}$$

Where:

$\%h^2_{bs}$: the broad sense heritability.
$\%h^2_{ns}$: the narrow sense heritability.
$\sigma^2 gca$: variance of general combining ability.
$\sigma^2 sca$: variance of specific combining ability.
$\sigma^2 e$: variance of experimental error of general and specific combining ability.
$\sigma^2 A$: variance of additive effect.
$\sigma^2 D$: variance of dominant effect.
$\sigma^2 G$: variance of grand genetic effect (additive and non-additive effects).
$\sigma^2 P$: variance of phenotypic effect.

- **Estimation of (ā)**

The estimation of (ā) was as follows:

$$\bar{a} = \sqrt{\frac{2\sigma^2 D}{\sigma^2 A}} = \sqrt{\frac{2\sigma^2 sca}{2\sigma^2 gca}} = \sqrt{\frac{\sigma^2 sca}{\sigma^2 gca}}$$

If the value of (ā) was:

0: this mean there is no dominancy.
1 > ā > 0: this mean there is a partial dominance.
1 : this mean there is a complete dominance.
1 < ā: this mean there is an over dominance.

- **Cluster Analysis**

The cluster analysis was performed through estimation the Euclidean Distance between trait means using the Nearest Neighbor method with aid of MVSP (Multi Variate Statistical Package, version 3.22) as follows (Technical Whitepaper, 2005):

$$d = \sqrt{\sum_{i=1}^{v} (p_{1i} - p_{2i})^2}$$

Where:

P_1 and P_2: the trait mean of the two individuals whose Euclidean Distance is to be measured, respectively.

3-2. Molecular Study

Genomic DNA extraction was conducted at the laboratories of Seeds test and certification office-Ministry of Agriculture. The molecular genetic analysis was accomplished in the Laboratory of Asco.-Baghdad Iraq. Apparatuses, Chemicals and Biological Materials were listed in Tables 2 and 3.

Table 2: Apparatuses names, manufacturing companies and origin used in the molecular study.

No.	Apparatus Name	Manufactured Company & Origin
1	Micro centrifuge	Thermo Scientific- USA
2	Electrophoresis unit	
3	Ultra violet transilluminator	Major Scientific-Taiwan
4	Gel documentation system	
5	Nanodrop	Biodrop-UK
6	Microwave Oven	Local market- China
7	Water bath	
8	Incubator	
9	Sensitive electronic Balance	
10	Deep freezer (-20 C°)	Teka- Spain
11	Cooling microfuge	
12	Micropipete	Eppendorf-Germany
13	Vortex mixer	Stuart Scientific-UK
14	Micro tubes	Promega-USA
15	PCR thermal cycler	BioRad- USA
16	Filter paper	Whatman-UK

Table 3: Names, manufacturing companies and origin of chemical and biological materials used in the molecular study.

No.	Chemical and Biological Materials	Manufacture Companies & origin
1	CTAB kit	Genetic engineering institute/Iraq
2	Geneaid kit	Geneaid Biotech Ltd.-China
3	Absolute ethyl alcohol	BDH-UK
4	Isoprobanol	
5	Agarose	Promega-USA
6	TE Buffer	
7	Ethidium bromide	
8	BSA	
9	Go Taq®Green master mix	
10	Loading dye	
11	Eco Adapter	Alpha DNA-Canada
12	H/M Adapter	
13	*EcoRI*enzyme	
14	*HpaII* enzyme	
15	*MspI* enzyme	
16	DNA ligase (T)	
17	*EcoRI*+A primer	
18	H/M+O primer	
19	(H/M) primer	
20	PCR markers (Lambda DNA *Pst*I Digest)	Sigma-USA

Table 4: Names and sequences of *EcoRI*, *HpaII* and *MspI* adaptors and primers used in the Pre-amplification reaction of MSAP.

Name	Sequence of Adaptors & Primers`3 → 5`
EcoRI Adaptor	CTCGTAGACTGCGTACC
H/M Adaptor	GATCATGAGTCCTGCT
Eco Pre-amplification primer	GACTGCGTACCAATTCA
H/M Pre-amplification primer	ATCATGAGTCCTGCTCGG

Table 5: Codes and sequences of H/M primers used in the final PCR reaction of MSAP.

Primer Code	Primers Sequences `3 → 5`
H/M-1	ATCATGAGTCCTGCTCGGTCT
H/M-2	ATCATGAGTCCTGCTCGGTCG
H/M-3	ATCATGAGTCCTGCTCGGTCC
H/M-4	ATCATGAGTCCTGCTCGGTTC

3-2-1. Extraction of the Total Genomic DNA

Fresh leaves samples from the 30 maize genotypes (Original and self-pollinated populations, each of 15 genotype) were collected at the 5-7 leaves seedling stage, and care was taken to ensure that the samples are free from pathogenic and insect infections. The samples were saved in a zipper bags and kept cold in an ice box and were transferred directly to the DNA extraction lab.

Total genomic DNA extraction was performed according to a modified procedure of CTAB (Genetic Engineering Institute-Baghdad-Iraq) and Genomic DNA Mini Kit - Plant (Geneaid Biotech Ltd., South Korea) protocols as follows:

- A hundred (100) mg of fresh leaves tissue were smashed with 700 µl of CTAB then the mixture was transferred to a 1.5 ml microfuge tube.
- RNase A was added in a volume of 5 µl to 200µl of GP1 (GP1 was mixed with RNase by gently pipetting).
- The RNase A and GP1 mixture were added to the microfuge tube that contains the extraction mixture then it was vortexed for 5 seconds.
- The extraction mixture was incubated at 60°C in a water bath for 10 minutes, and tubes were inverted every 5 minutes. At this step, the elution buffer (100 µl for each sample) was incubated at 60°C in the water bath.
- GP2 was added in a volume of 100 µl to each sample and vortexed for 5 sec. then it was placed on ice for 3 min.
- The solution was transferred to 1.5 ml filter column tube.
- The tube (with filter column) that contains the solution was spun by the microfuge at 8000 g for 1 min.
- The filter column was discarded and the supernatant was transferred to a new 1.5 ml tube.
- GP3 and isopropanol mixture were added in a volume of 1.5 times of the supernatant then the mixture was vortexed for 5 sec.
- A 700 µl of the supernatant was transferred into GD column tube then spun by microfuge (13000 rpm) for 2 min.
- The GD column was removed and the supernatant was discarded, then the GD column was put back again to the same tube. So, the rest of supernatant was transferred to the same GD column tube, and centrifugation step was repeated under the same conditions.

- The supernatant was discarded and the GD column (which contains DNA sample) was placed in a new 1.5 ml microfuge tube.
- The W1 solution was added in a volume of 400 µl and spun at 13000 rpm for 30 sec.
- A volume of 120 µl of washing buffer with 480 µl of ethanol were added then spun at 13000 rpm for 30 sec.
- The supernatant was discarded and the filter column was put back in the same tube then spun at 13000 rpm for 30 sec. to dry the column.
- The filter column (which contains DNA) was transferred to a new 1.5 ml tube and 100 µl of the elution buffer (which already has been incubated in water bath at 60°C) was added to each tube. It was left for 3-5 min to absorb the entire elution buffer then it was spun at 13000 rpm for 30 sec. Finally, the filter column was discarded and the supernatant that contains DNA was kept in the refrigerator (-4°C).

3-2-2. DNA Quantification and Purity

The validity of the extracted DNA was checked by mixing 3µl from each DNA sample with 7µl loading buffer and running on a 1 % agarose gel.

The purity and quantity of DNA samples were checked individually with nanodrop. Reads ranged 1.8-2, as stated in the following formula:

$$\textit{Purity of DNA} = O.D_{260} / O.D_{280} = \geq 1.8$$

The variation in the DNA concentration was adjusted with TE buffer to a final concentration of 50 ng/µl for PCR amplification.

3-2-3. Preparation of MSAP Restriction Enzymes

Three of MSAP specific restriction enzymes, which were *EcoRI*, *HpaII* and *MspI* were supplied by Promega company (Promega-Madison,

Wisconsin- USA) and the supplier instruction was followed to restrict the DNA samples as follows:

- The enzymatic restriction mixture of *MSPI+EcoRI* enzymes was prepared by adding 1 µl from each enzyme with 2µl of its corresponding buffer plus 0.2 of bovine serum albumin (BSA), and D.D. water was added in a volume of 7.8 µl.
- The restriction enzymatic mixture *HpaII+EcoRI* was prepared in the same way.
- The DNA sample was added to the restriction mixture with a volume of 6 µl.
- The mixture was centrifugated for 5 sec. then it was incubated at 37 C° for three hours.
- T4 DNA ligase was prepared by mixing 6µl of D.D. water with 1 µl of T4 enzyme and 1 µl of T4 buffer plus 1 µl from each Eco-adapter and H/M adapter.
- Samples were incubated overnight at 37 C° in the incubator.
- A volume of 5 µl of T4 ligase was added to each of the microfuges.
- The final mixture was diluted in 1:10 ratio by adding 10 µl from the mixture to 90 µl of TE buffer in a new microfuge tube, then it was centrifuged for 5 sec.

3-2-4. Pre-Amplification Reaction

Pre amplification reaction was performed as in the following steps:

- Distilled De-ionized water (D.D. water) was added in a volume of 3.5 µl in 1.5 ml microfuge tube.
- Ten microliters (10µl) of master mix were added to each tube.

- From each of *EcoRI*+Aand *H/M*+O primers, 7 µl were added to each tube.
- The tubes were centrifuged for 5 sec., then they were placed in PCR thermal cycler, and the thermal profile was as listed in the table.

Table 6: Thermal profile of pre-amplification reaction.

Steps	Temperature C°	Time	No. of Cycles
In. Denaturation	95	5 min.	1
Denaturation	95	30 Sec.	30
Annealing	56	1 min.	
Extension	72	1 min.	
Final Extension	72	7 min.	1

3-2-5. Final Amplification Reaction

The final amplification reaction was as follows:

- The PCR product in the pre-amplification step was diluted with 1:20 ratio by adding 5µl of the pre-amplification product to 95µl of TE buffer.
- Distilled deionized water was added in a volume of 3µl in a new microfuge tube.
- A volume of 10 µl of mastermix was added to each tube.
- A volume of 1 µl of each EcoRI and H/M was added to each tube.
- Then, 5 µl of DNA was added and centrifuged for 5 sec., then it was placed in the thermal cycler programmed on the following thermal profile:

Table 7: Thermal profile of the final amplification reaction.

Steps	Temperature C°	Time	No. of Cycles
In. Denaturation	95	5 min.	1
Denaturation	95	30 sec.	12 cycle
Annealing	65	30 sec.	
Extension	72	1 min.	
Denaturation	95	30 sec.	23 cycle
Annealing	56	30 sec.	
Extension	72	1 min.	
Final exten.	60	30 min.	1
Hold	4	----	----

3-2-6. Electrophoresis

Agarose gel was prepared by dissolving 2 g of agarose powder in 100 ml of 1x TBE buffer. The mixture was heated in microwave till the agarose powder completely dissolved. It was left on the bench to cool down then ethidium bromide was added in a final concentration of 0.5 µg ml^{-1}. The plate was tightly surrounded with tape to make sure there are no leaks. The comb was stabilized in the plate slide, then the melted agarose was added carefully to the plate and left to solidify. After a while, the comb and the tape were removed. The plate and the solidified agarose gel over they were transferred to a tank which contains a volume of 1x TBE buffer sufficient to submerge the gel plate.

Ten microliters (10 µl) were taken from each PCR product and loaded into the sample wells. Electrophoresis was done at a voltage of 5 volt cm^{-1} for 45 min. till DNA samples reached the edge of the gel. The agarose gel was transferred to a UV Transilluminator, photographed at 340 nm and documented with gel documentation system.

3-2-7. Statistical Analysis of MSAP Data

MSAP results in the final PCR step were converted into a binomial matrix (0-1) in a form of excel worksheet. It was allotted with number 0 and 1 to the absence and the presence of a fragment respectively, in the results of H/M isoschizomers. Therefore, there will be two reads for each sample as (0-1) binary. Excel sheet was converted to CSV form (Comma Separated Values). R statistical package (Version 3.3.2) with aid of MSAP package (Version 1.1.9) was used to analyze the MSAP results which were converted into CSV form. Principal Coordinates Analysis (PCoA) and Shannon's Index were estimated. Cluster analysis for MSAP results was conducted through estimating of Euclidean Distance according to the nearest neighbor.

Analysis of Molecular Variance (AMOVA) was performed as follows (Excoffier, 2001):

Table 8: Analysis of Molecular Variance (AMOVA) of Methylation Sensitive Loci (MSL) using MSAP technique.

Source of variance	df	Sum of squares	Mean squares	Variance
Among demes within groups	$d - G$	$SS(AD) = \sum_g \frac{1}{2n_g} \sum_i \sum_{i'} \sum_j \sum_k \delta^2_{jk}$ _ $SS(WD)$	$\frac{SS(AD)}{d-G}$	$\sigma^2_{AD} = \sigma^2_{WG} + n'\sigma^2_b$
Among groups within population	$G - 1$	$SS(AG) = SS(T) - \sum_g \frac{1}{2n_g} \sum_i \sum_{i'} \sum_j \sum_k \delta^2_{jk}$	$\frac{SS(AG)}{G-1}$	$\delta^2_{WG} = \frac{SS(AG)}{G-1}$
Total	$n - 1$	$SS(T) = \frac{1}{2n} \sum_j \sum_k \delta^2_{jk}$		$\sigma^2 = \sigma^2_a + \sigma^2_b + \sigma^2_c$

4. Results and Discussion

4-1. Molecular Analysis of MSAP Data

4-1-1. Amplification of Restricted Loci

The molecular results indicated that the used four primers succeeded in identifying a total of 39 specific sites resulted from using of *HpaII* and *MspI* methylation sensitive enzymes (table 9). Thirty out of these loci have been described as Methylation Sensitive Loci (MSL), while the rest (9 loci) were defined as No Methylated Loci (NML). Around half of the MSL (16 loci) was found to be polymorphic, hence achieving polymorphism percentage of 53%. Approach results were detected for the nine NML as five of them were identified to be polymorphic and scored a polymorphism percentage of 56%. This fact that MSL were much varied compared with NML is supported by Shannon's index which responds positively to the variations expressed by MSL and NML to score 0.56 and 0.26, respectively. Many results from different studies were supportive to the present outcomes (Eichten et al. 2013; Almelhami, 2017).

Table 9: The number and the type of restricted loci resulted from using *HpaII* and *MspI* methylation sensitive enzymes with four MSAP primers in the original and self-pollinated maize populations.

Primer	Loci/Primer	Loci Type		Polymorphic Loci		Polymorphism%		Shannon's Index	
		MSL	NML	MSL	NML	MSL	NML	MSL	NML
H/M1	10	6	4	5	3	83	75	0.54	0.23
H/M2	10	9	1	7	1	78	100	0.59	---
H/M3	11	9	2	3	1	33	50	0.57	0.22
H/M4	8	6	2	1	0	17	0	0.52	---
Total	*39*	*30*	*9*	*16*	*5*	*53*	*56*	*0.56*	*0.26*

The four used H/M primers have differed in their ability to identify the restricted loci. The first primer HM1 (Figure 2) was able to characterize 10 loci, 6 out of them were MSL, and the rest (4) were NML. Five and three out of MSL and NML loci respectively, were polymorphic. Accordingly, the polymorphism percentage of MSL was a little bit higher (83%) than the NML (75%).

Similar findings were extracted from using the second primer H/M2 (figure 3) as it produced 10 loci. The majority of these loci (9) were described as MSL mean, while, 7 of these MSL were polymorphic. The seven polymorphic MSL loci recorded a polymorphism percentage of 78%. On the other side, the only one NML locus was polymorphic and thus scoring 100% polymorphism percentage.

The H/M3 primer (figure 4) was the most efficient primer with 11 amplified loci. Three of the 9 identified MSL loci were found to be polymorphic, consequently the polymorphism percentage was one-third of the estimated value (33%). The NML loci were just 2, half of which was polymorphic and this was enough to achieve 50% polymorphism.

The forth primer (figure 5) exposed a very modest performance when it produced only 8 loci. The MSL and NML loci were 6 and 2, respectively. Form the detected MSL loci, only 1 was polymorphic and at the same time the NML was so common to show up in all the studied genomes, therefore the polymorphism percentage was zero.

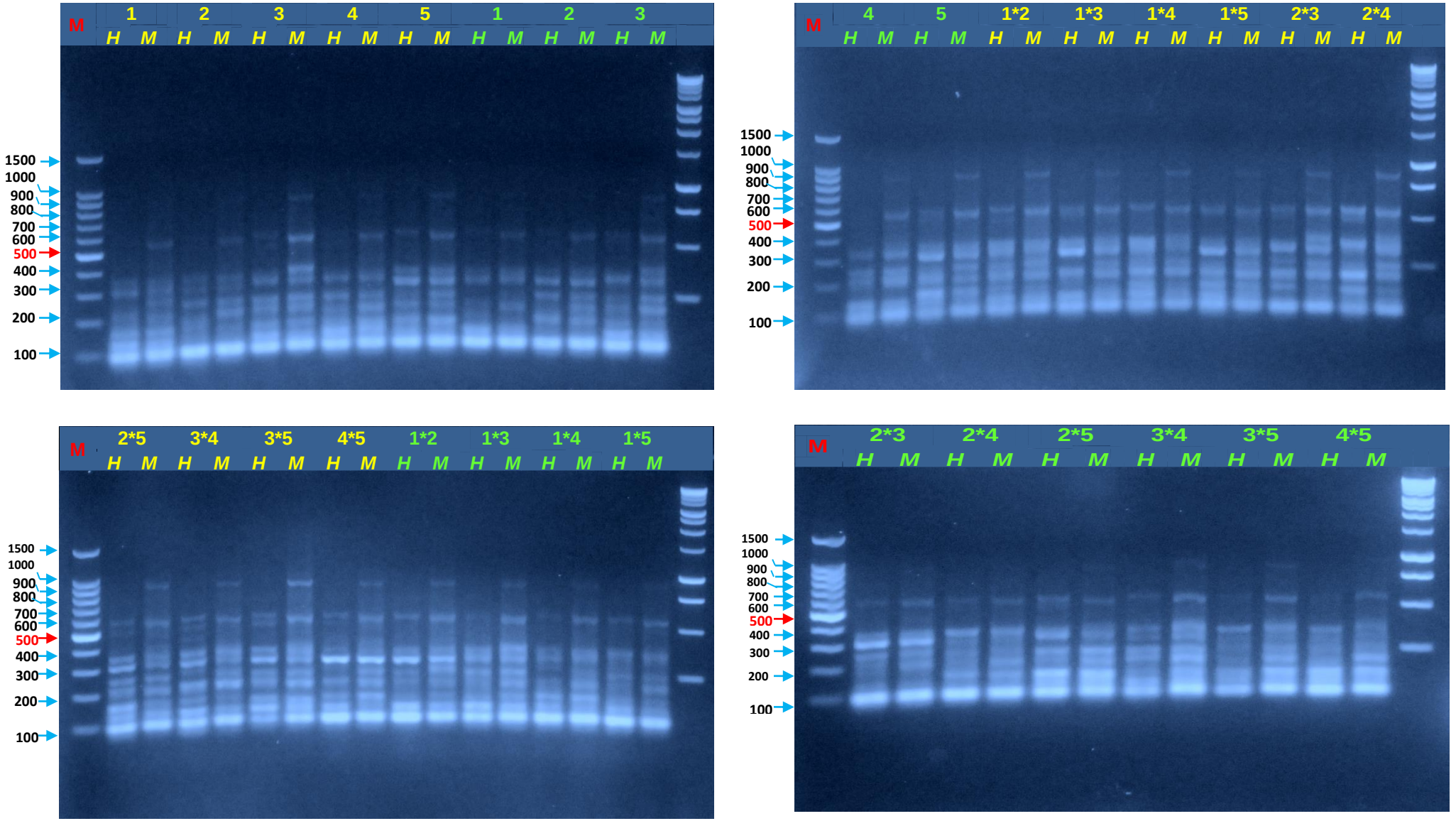


Figure 2: The amplified loci restricted by *HpaII* and *MspI* enzymes in the genomic DNA of original (yellow) and self-pollinated (green) populations of maize using H/M1 primer in MSAP technique. M=Marker

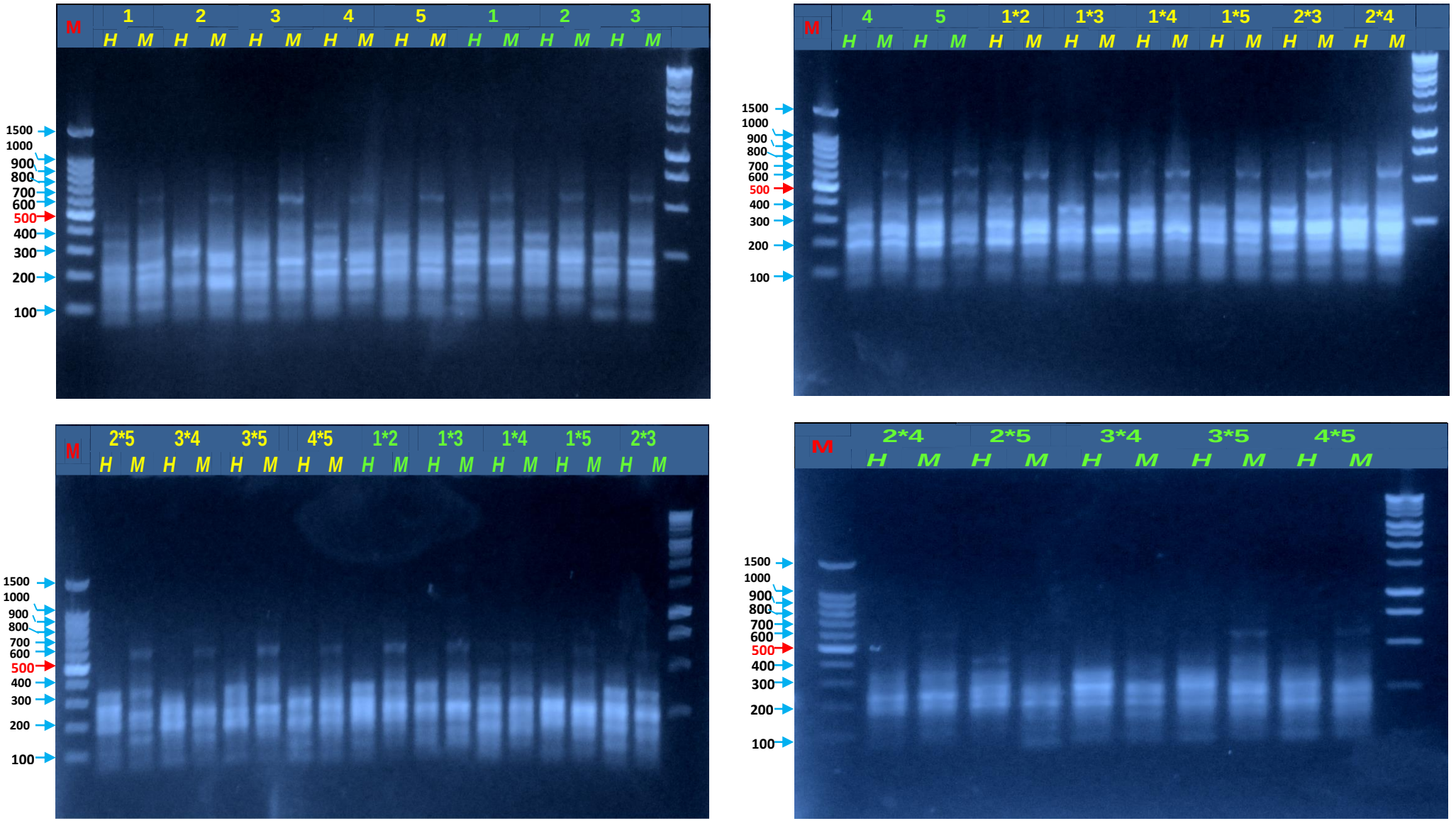


Figure 3: The amplified loci restricted by *HpaII* and *MspI* enzymes in the genomic DNA of original (yellow) and self-pollinated (green) populations of maize using H/M2 primer in MSAP technique. M=Marker

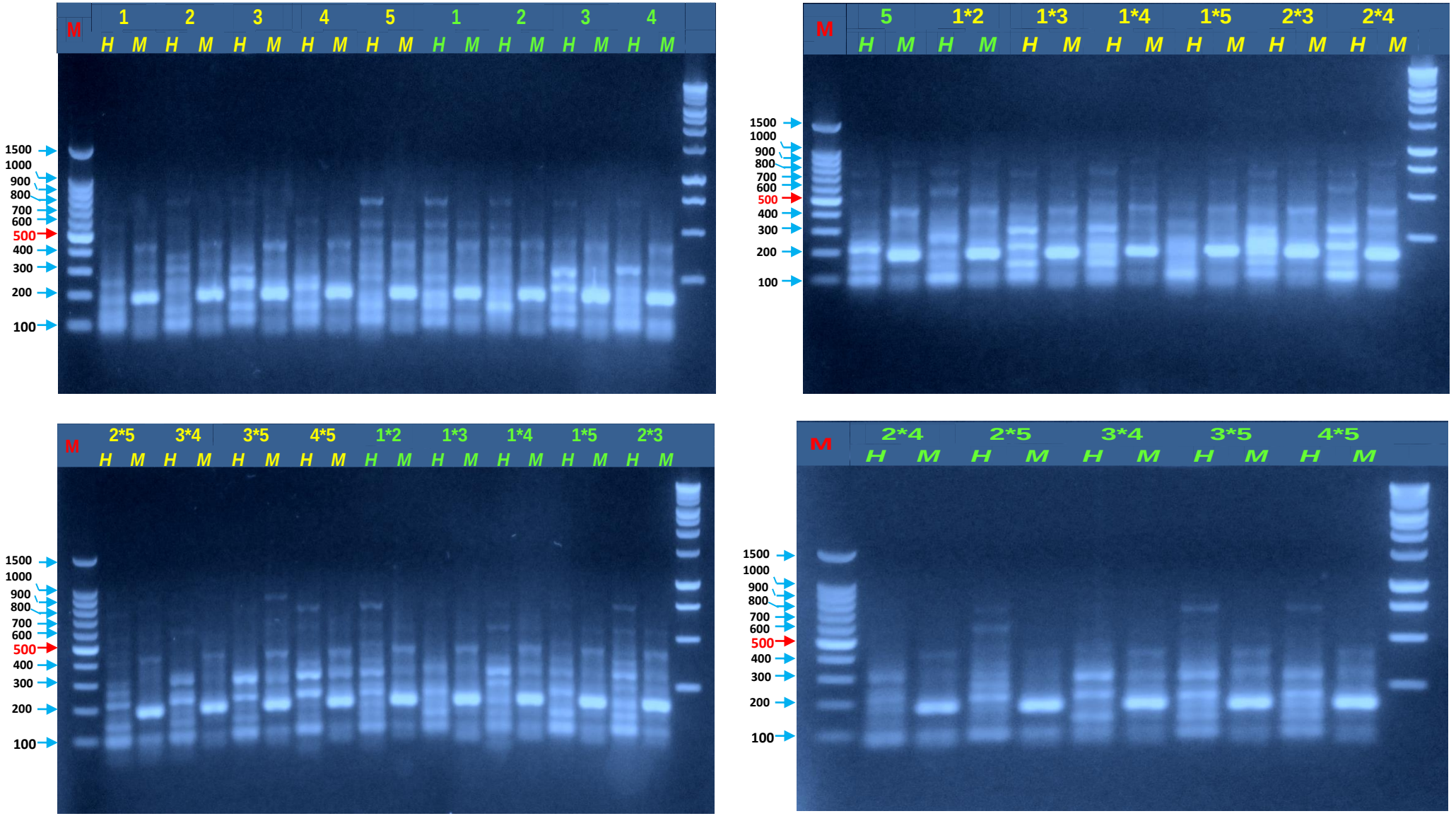


Figure 4: The amplified loci restricted by *HpaII* and *MspI* enzymes in the genomic DNA of original (yellow) and self-pollinated (green) populations of maize using H/M3 primer in MSAP technique. M=Marker

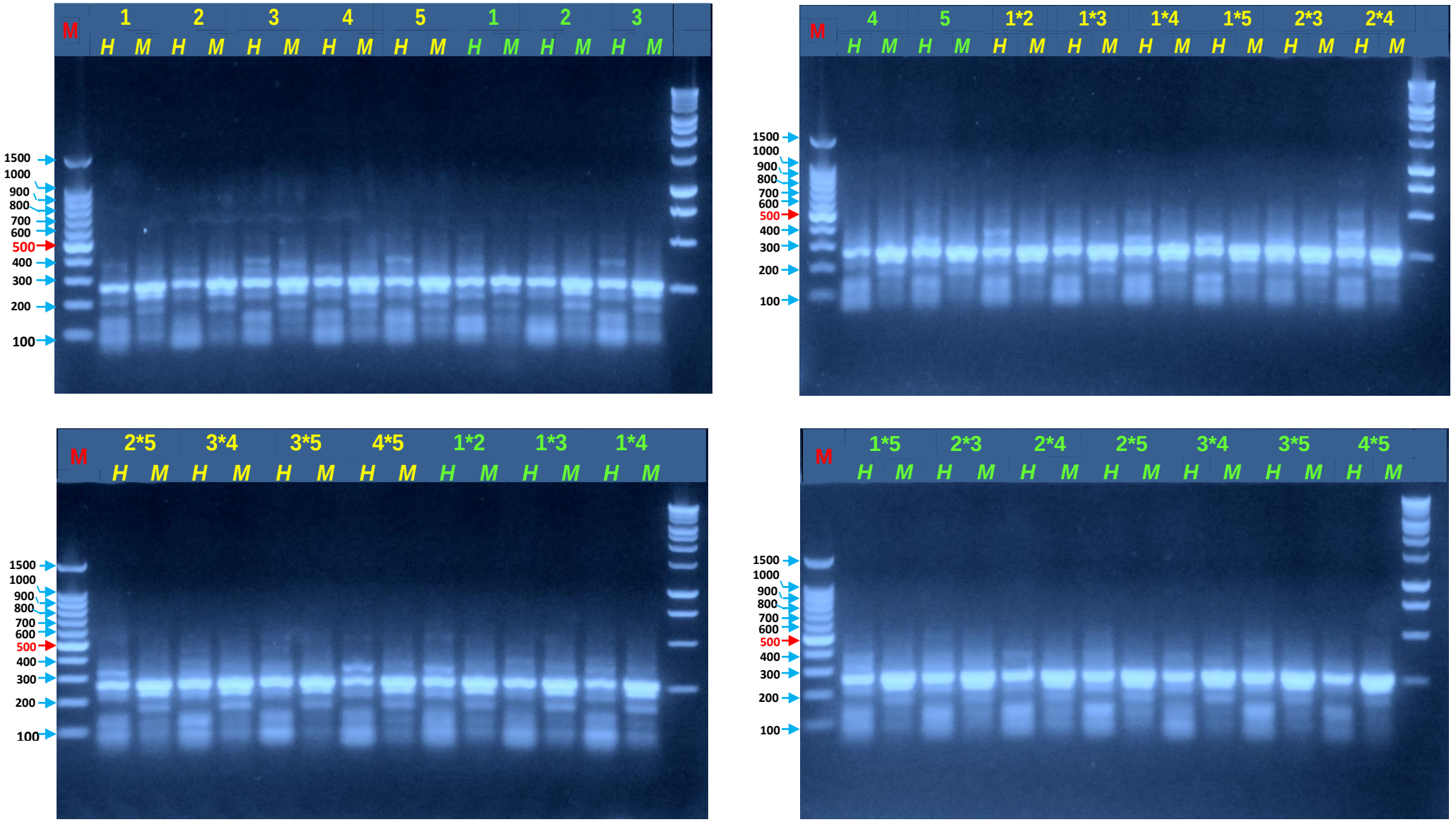


Figure 5: The amplified loci restricted by *HpaII* and *MspI* enzymes in the genomic DNA of original (yellow) and self-pollinated (green) populations of maize using H/M4 primer in MSAP technique. M=Marker

4-1-2. Methylation Status

The results of analysis of molecular variance (AMOVA) presented in Appendix 1 revealed a significant difference in the differentially methylated regions (DMRs) spreading within all the studied genomes by using four MSAP specific primers. The DNA methylation status (table 10) cleared that the studied genotypes showed different levels of variation, consider each state in response to the homozygosity and inbreeding level, as well as the hybridization impact.

The previously mentioned table (10) illustrated the differences of methylation status between the different genotypes represented in couples. In the first couple, the self-pollinated and original parental lines were compared against each other. Two rounds of selfing did not affect the unmethylated state of the genomic DNA, as both, the original and self-pollinated inbreds had the same number of unmethylated regions (22.7%).

On the other hand, a gap of 0.7% was detected in the hemimethylated regions between the self-pollinated lines and their original counterparts, as the first showed the minimum value (27.3%) compared with the maximum value (28.0%) achieved by the second. The variation of the internal cytosine methylation was significantly magnified due to the selfing process and it reached 26.0% compared with its original inbreds that gained the lowest percentage (22.7%). Furthermore, the fully methylated regions integrated with the genomic DNA of the original lines were less than what was existed in the self-pollinated inbreds (26% and 24%, respectively). This represents a clear indication of the inbreeding importance in modifying epigenetic changes, which in turn will play a crucial role in the phenotypic variations. These findings indicate a rise in

the methylation level in the self-pollinated inbreds, which may affect the quantitative performance of inbreds per se and their hybrids. The current results agree to some extent with those established by Feng et al. (2015).

The other comparison consisted of self-pollinated and the original hybrids, through which we may comprehend the role of hybridization in the alteration of DNA methylation in the original inbreds and two selfing generations later. The results indicated that there is only a slight deviation in the level of the unmethylated status in favor of the self-pollinated hybrids against their original copy. The effect of self-pollination was different in the percentage of hemimethylated regions when it decreased from 26.8% to 25.5% after two generations of selfing. The state of internal cytosine methylation has heightened in the self-pollinated hybrids to become 23.6% after it was 21.6% in the original copy. The full methylation state was on the opposite direction when it scored a higher value in the self-pollinated hybrids compared with the original hybrids. It's so obvious that the differences in the methylation status of both, self-pollinated and original parental lines were transmitted in the same manner to their diallel hybrids. Gutzat and Scheid (2012) and Lauria et al. (2014) pointed to similar findings and to the great importance of stable or semi stable inherited patterns of DNA methylation.

At the next level, the comparison was settled within each group of parental lines and their half diallel hybrids to trace the probable effect of hybridization in the distribution of methylation domains (table 10). It seems that it is not easy to trace the effect of hybridization in the self-pollinated population due to values convergence. No differences were detected between inbreds and their F1 dialleles in the unmethylated regions. In the case of hemimethylated and internal cytosine methylation regions, parents have higher values (27.3% and 25.3%) compared with

their descended hybrids (26.7% and 24.0%), respectively. These results are fairly logical as hybrids genomes are usually described to have a higher level of gene expression due to less susceptiblity to limitations like DNA methylation. However, hybrids act in a different way as they show a ratio of fully methylated status higher than their parents (23.3% and 21.3%, respectively), even so this may be attributed to the absence of enzymes targets. The present results have shared the same findings with previous studies (Liu et al., 2014).

The next comparison will be similar to the previous, but it will be within the original population. Slight differences have been characterized between the original lines and their single hybrids corresponding unmethylated (20.0% and 20.7%) and hemimethylated regions (31.1% and 30.4%), respectively. At the same time, the results of the epigenetic assessment of the other two states, internal cytosine methylation and fully methylated regions which were completely matched (24.1%) confirmed the neutral role of hybridization in the redistribution of methyl groups along the genomic DNA.

The last comparison represents the total performance of both original and self-pollinated populations (inbreds and half diallel). The results were a little bit confused because each group of the parental lines acted in a different way via hybridization. For instance, at the same time as the percentage of unmethylated regions increased from 26.2% in the original population to 27.1% in the self-pollinated population, the hemimethylated regions decreased from 27.6% to be 26.4% after two selfed generations. The regions of methylated internal cytosine increased with about 2% at the next generations to reach 24.4% in the DNA of the self-pollinated pop., while the fully methylated regions decreased to become 22% after it was 23.8% in the DNA of the original population.

Table 10: Methylation status (%) of the compared maize populations using four MSAP primers.

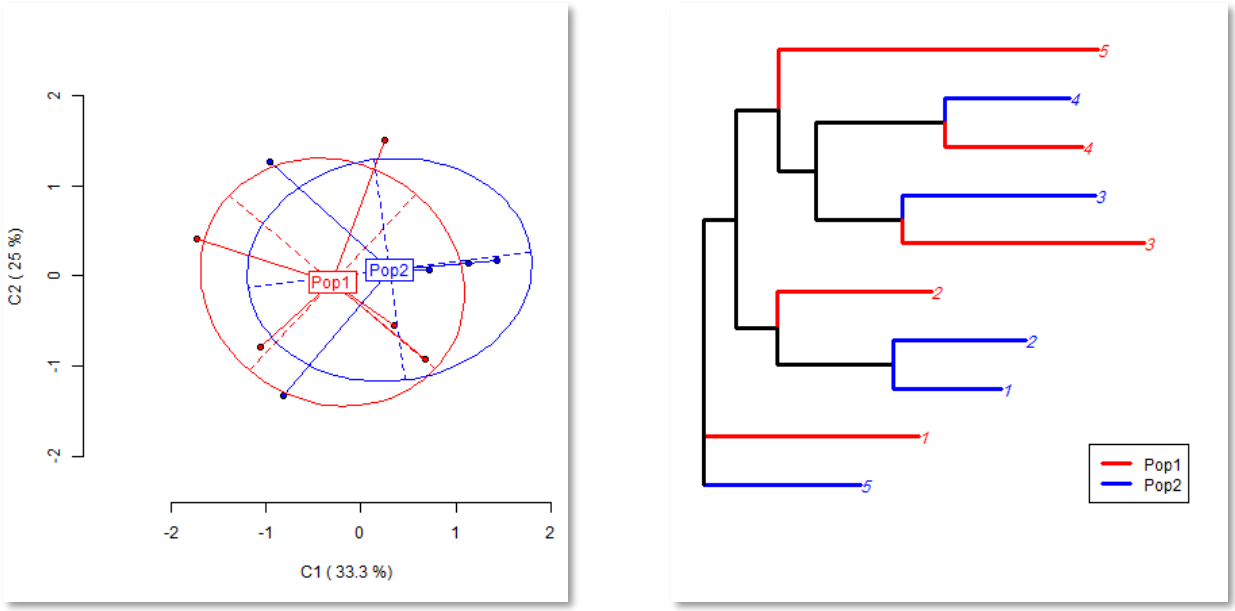
Compared Populations		Methylation Status %			
		Unmethylated	Hemimethylated	Internal cytosine methylation	Full methylation or absence of target
		HPA+/MSP+	HPA+/MSP-	HPA-/MSP+	HPA-/MSP-
Self-poll. Lines vs. Original Lines	Self-poll. Lines	22.7	27.3	26.0	24.0
	Original Lines	22.7	28.0	22.7	22.7
Self-poll. Hyb. vs. Original Hyb.	Self-poll. Hyb.	28.4	25.5	23.6	22.6
	Original Hyb.	28.1	26.8	21.6	23.6
Self-poll. Lines vs. Self-poll. Hyb.	Self-poll. Lines	26.0	27.3	25.3	21.3
	Self-poll. Hyb.	26.0	26.7	24.0	23.3
Original Lines vs. Original Hyb.	Original Lines	20.0	31.1	24.4	24.4
	Original Hyb.	20.7	30.4	24.4	24.4
Self-poll. Pop. vs. Original Pop.	Self-poll. Pop.	27.1	26.4	24.4	22.0
	Original Pop.	26.2	27.6	22.4	23.8

4-1-3. Principal Coordinates (PCoA) and Cluster Analysis for MSAP Data

The results of principal coordinates and cluster analysis (Figure 6; A and B) of Methylated Sensitive Loci (MSL) and Non-methylated Loci (NML) pointed to distinct variation in the pattern of DNA methylation and the distribution of DMRs within and between populations (original and self-pollinated inbreds). The first comparison of Methylation Sensitive Loci (MSL) was made between the original inbred lines and their self-pollinated copy after two generations.

According to the results of Principle Coordinate Analysis (PCoA), the compared populations recorded a higher variation via the first coordinate (C1) reached 33.3%, while the second coordinate (C2) showed less variation (25%). Figure (6) of the cluster analysis revealed that the inheritance of MSL has differed from one inbred to another. For instance, the original inbreds 3 and 4 maintained substantially their epigenetic performance when they sticked togather with their self-pollinated counterparts and occupied the same sub sub-cluster after two rounds of selfing. Some of the self-pollinated inbreds such as 1 and 5, acted in a different way as they situated far away from their original parents, hence located in different clusters. The possession of inbreds for such a large epigenetic variation will serve in widening the total genetic gap, especially when it associated with the genetic variation and thus increases the chance of obtaining hybrid vigor in the desired direction.

Inbred 2 was between the two previously described states as the self-pollinated inbred separated from its original parent, however, they still belonged to the same sub-cluster (Figure 6).



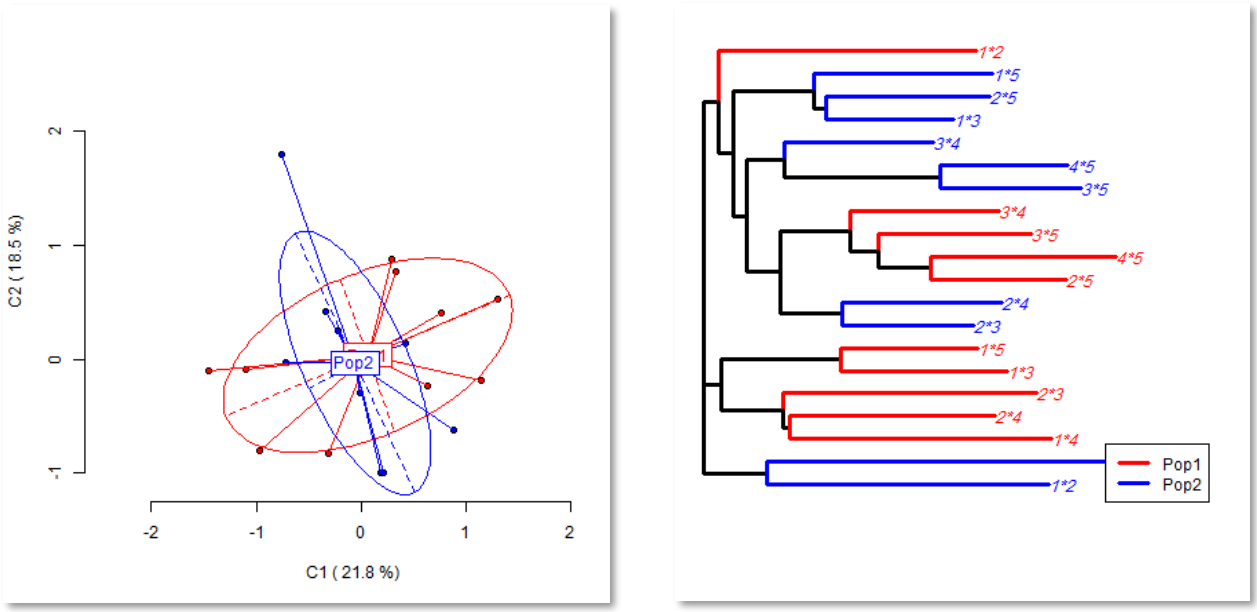
(A) (B)

Figure 6: (A) The Principal Coordinates Analysis (PCoA), and (B) the hierarchical clustering using the nearest neighbor method for the Methylated Sensitive Loci (MSL) in the five original (Blue) and their self-pollinated inbred lines (Red) of maize.

The other comparison (Figure 7) was settled between the original and the self-pollinate hybrids. The coordinates cleared that the diallel hybrids were more homogenous and showed less variation compared with their inbred parents. The hybrids varied at the first coordinate (C1) with 21.8%, while the second coordinate (C2) varied with 18.5%. The original hybrids 1x2 and 1x4 were the most deviant of the rest of the original hybrids, hence, they formed one cluster (Figure 7). After two rounds of selfing, the 1x4 hybrid was highly affected and returned to join its population, while the other hybrid (1x2) preserved his unique performance to occupy one cluster alone.

The previous overview brightens up the tendency of the self-pollinated hybrids to form distinct pools and distance themselves from their original

parents. The diallel hybrids (original and self-pollinated) were alienated into six distinct groups. The first group was merely composed of the self-pollinated hybrid 1x2, while the second group was composed of six original hybrids (1x5, 2x5, 1x3, 3x4, 3x5 and 4x5). Four members belonged to the third group, all were self-pollinated hybrids (3x4, 3x5, 2x5 and 4x5), whereas the fourth group was built-in just one pair of the original hybrids (2x3 and 2x4) that has a common parent. The self-pollinated hybrids 1x3, 1x4, 1x5, 2x3 and 2x4 joined together to form the fifth group. Finally, the two original hybrids 1x2 and 1x4 showed unique MSL pattern, hence they occupied the same main cluster.

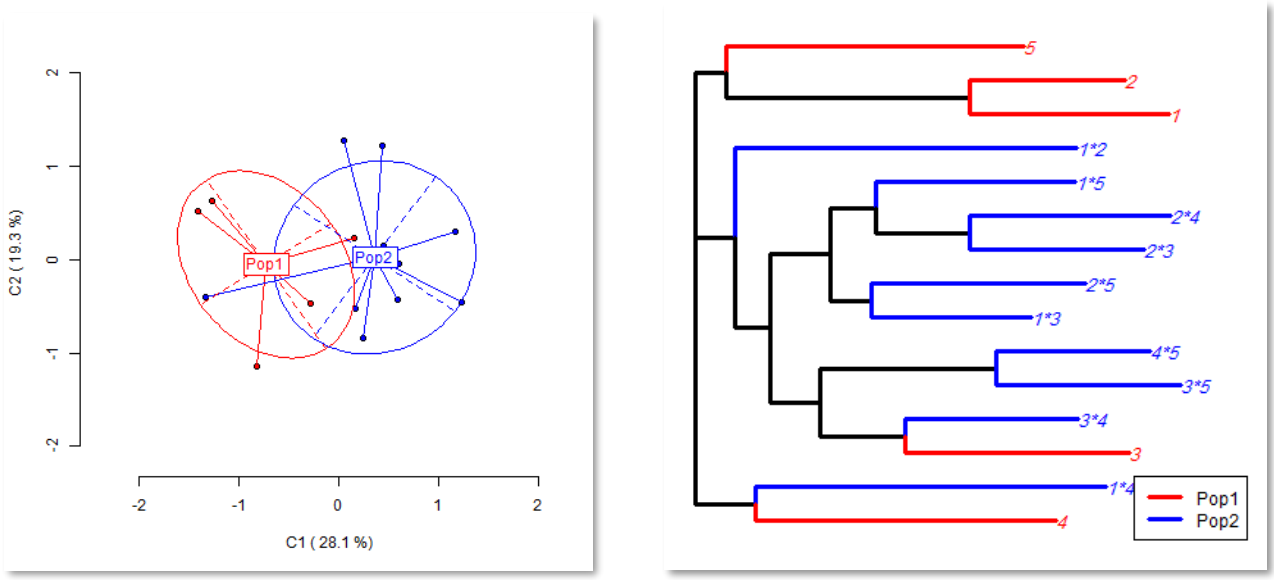


(A) (B)

Figure 7: (A) The Principal Coordinates Analysis (PCoA), and (B) the hierarchical clustering using the nearest neighbor method for the Methylated Sensitive Loci (MSL) in the ten original hybrids (Blue) and their self-pollinated counterparts (Red) of maize.

In an attempt to investigate the impact of selfing on the epigenetic structure of maize inbreds and their diallel hybrids, the variations in the methylation sensitive loci (MSL) were analyzed (Figure 8; A and B). The principal coordinates analysis of the original population revealed that the size of the difference at the first coordinate (C1) has reached 28.1%. At the same time, this population showed less variation at the second coordinate (C2), (19.3%). With regard to MSL, there is a great variation between the original inbreds and their F1 hybrids when each has its own center and thus pointing to the large role of hybridization in changing the pattern of distribution of MSL. Almelhami (2017) stated results close to what is currently on the table.

Based on MSL results in the original population, cluster analysis approved the aforementioned results (Figure 8). Some inbreds like 3 and 4, succeeded in transmitting their MSL pattern to their progeny (3x4 and 1x4 hybrids, respectively) which serve them to share the same cluster. On other occasions, the radical change of the F1 epigenetic behavior reinforced the view that the hybridization may lead to widespread genetic and epigenetic disturbance.

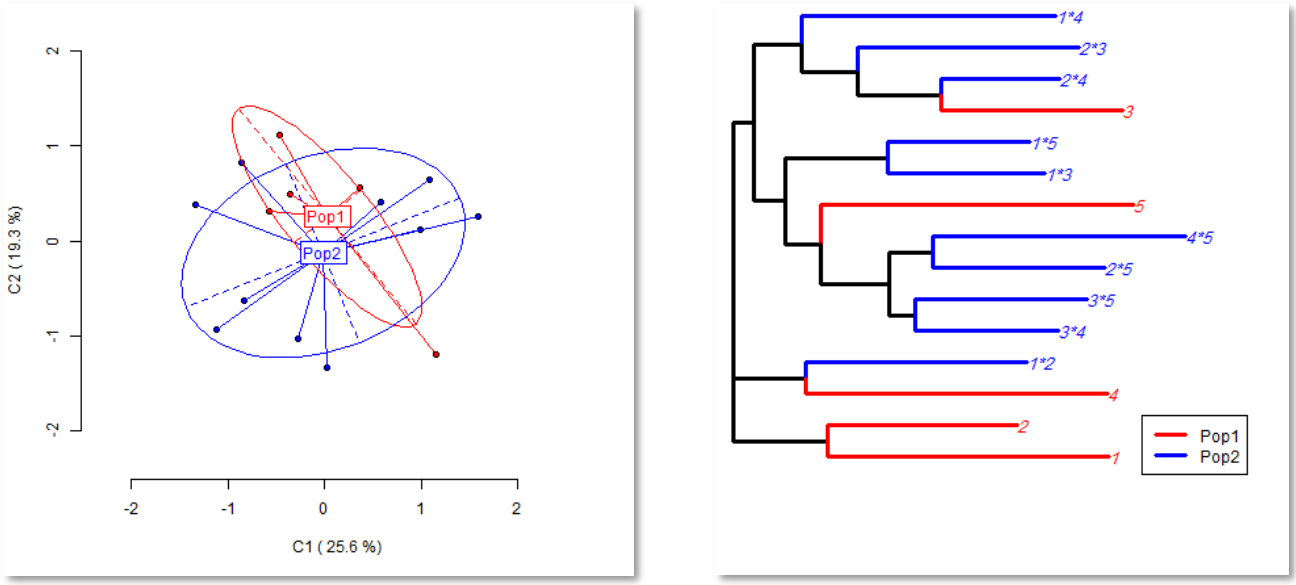


(A) (B)

Figure 8: (A) The Principal Coordinates Analysis (PCoA), and (B) the hierarchical clustering using the nearest neighbor method for the Methylated Sensitive Loci (MSL) in the five original inbreds (Red) and their half diallel hybrids (Blue) of maize.

After two generations of selfing, the pattern of the Methylation Sensitive Loci (MSL) within the self-pollinated population (Inbreds against their diallel hybrids) has been re-evaluated. The first coordinate (C1) in the principal coordinates analysis scored a variation value (25.6%) higher than the second coordinate (C2), (19.3%). These outcomes indicated that the inbreds and their half diallels were more homogenous compared with the original copy (Figure 9; A).

The self-pollinated population was splited into 4 groups (Figure 9), each has parental and F1 members. Interestingly, the two inbreds 1 and 2 remained side by side to form the same cluster, with inbred 1 being the most epigenetically distant.



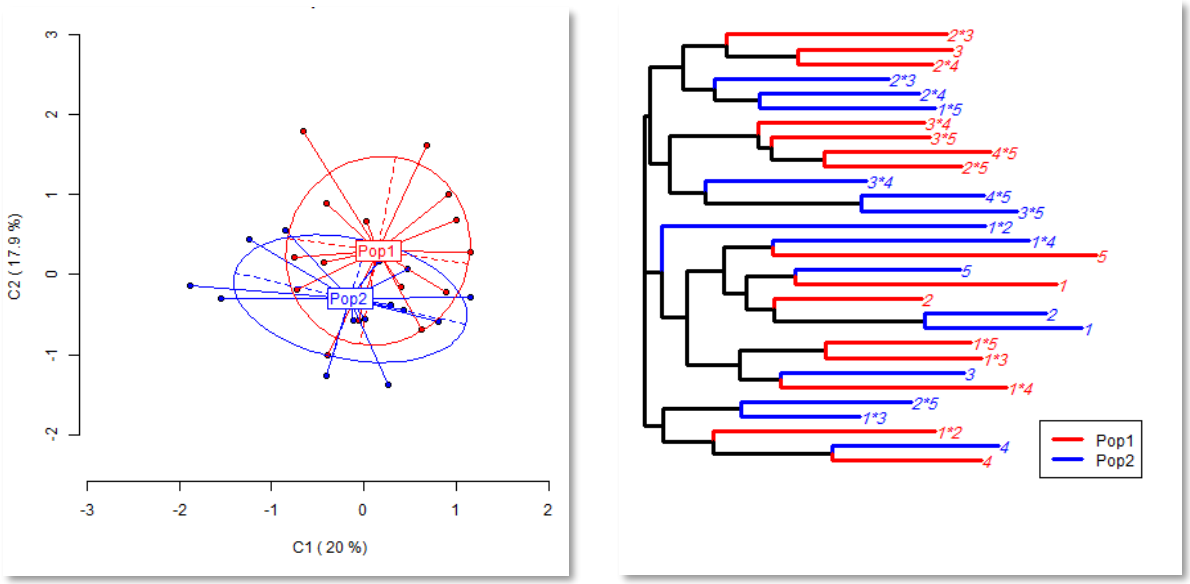
(A) (B)

Figure 9: (A) The Principal Coordinates Analysis (PCoA), and (B) the hierarchical clustering using the nearest neighbor method for the Methylated Sensitive Loci (MSL) in the five self-pollinated inbreds (Red) and their half diallel hybrids (Blue) of maize.

The last comparison was made between the original population (inbreds and their diallel hybrids) and the self-pollinated population (inbreds and their diallel hybrids). It seems clear through Figure 10 (A and B) that the two populations have shown a vast area of dispersion and neither has maintained their separate group, this can be due to their implicit differences as parents against their respective hybrids.

The principal coordinates analysis revealed that the first coordinate (C1) recorded variation about 20%, which decreased at the second coordinate (C2) to be 17.9%. From the cluster analysis of MSL data (Figure 10), it can be noticed that the entire genotypes were arranged in 4 clusters plus the unique cluster occupied by 1x2 self-pollinated hybrid. The epigenetic distinctness of the original parental inbred 5 was so clear, as well as its counterpart number 1.

Despite the obvious effect of the selfing process in the majority of the studied genotypes, the original and the self-pollinated copies of inbred 4 still show remarkable epigenetic stability which drove them to share the same cluster. Nevertheless, the self-pollinated copy of inbred 4 had a more heterogeneous tendency compared with the inbred that it descended from.



(A) (B)

Figure 10 : (A) The Principal Coordinates Analysis (PCoA), and (B) the hierarchical clustering using the nearest neighbor method for the Methylated Sensitive Loci (MSL) in the original (Red) and the self-pollinated populations (Blue) of maize.

4-2. Quantitative Genetic Analysis

4-2-1. Hybrid vigor

4-2-1-1. Anthesis days (day)

The analysis of variance revealed significant differences within both populations (original and self-pollinated) for the anthesis trait (Appendix 2). The earlier inbred from the original population (table 11) was line 1 as it spent 53.67 days to anthesis. By contrast, 57 days were hardly enough for line 5 to anthesis, so it was the latest. However, this duration was not enough for the same inbred lines to shed their pollens after two rounds of selfing (table 12), where, the latest inbred (line 3) required 59.33 days. Although, inbred 1 maintained its early flowering, it was slightly retreated compared with its ancestor (original inbred 1) as it spent 56.33 days. In essence, these results assured the negative effect of self-pollination in the earliness of anthesis which may be attributed to inbreeding depression.

The original inbreds have successfully passed down the variation in the anthesis time to their hybrids as the latest differed significantly too (table 11). Quite remarkably, the original hybrids 1x2 and 1x3 gave the lowest mean for this trait (51.67 days), whereas the highest which was 56.67 days was shown by the hybrid 4x5. Seven out of the ten generated original hybrids exposed significant negative hybrid vigor against their earliest parent with a maximum percentage of -4.85% for 2x4 (table 11). These negative values reflected the over dominant type of gene action of the earliest parent expressed in such crosses.

The grand mean of self-pollinated hybrids (table 12) which was 52.93 days in contrary with the grand mean of their parents (57.93 days)

showed the improved performance regarding anthesis time after two generations of selfing. However, the superiority of F1 hybrids over their best parent was additional evidence for the self-pollination effect. It can be observed that 1x5 and 2x5 hybrids were the earliest with 51.33 days until anthesis (table 12). While the latest was the hybrid 4x5, even so, it required a shorter duration (55 days) than its original inbred.

The inheritance of anthesis time was strictly regulated by over dominance genes of the early parent after two generations of selfing (table 12) since all the self-pollinated hybrids marked with negative hybrid vigor, and 3x5 hybrid was in the forefront (-11.42%). The significance of positive and negative hybrid vigor has been identified in previous studies (Al-Falahy, 2015; Abdul-Hamed et al. 2017) that have agreed to some extent with what was reached in the present study.

Table 11: Means of anthesis (day) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	53.67	51.67	51.67	53.00	54.00
2	-3.73	55.00	53.67	52.33	53.00
3	-3.73	-2.42	56.67	55.33	56.33
4	-1.25	-4.85	-2.36	56.67	56.67
5	0.61	-3.64	-0.59	-0.01	57.00
Grand Mean of Parents		55.80	Grand Mean of Hybrids		53.77
LSD 5%	1.40	SE	0.88		

Table 12: Means of anthesis (day) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	56.33	54.33	52.67	52.33	51.33
2	-3.54	57.33	52.67	54.00	51.33
3	-6.50	-8.13	59.33	54.00	51.67
4	-7.10	-5.81	-7.42	58.33	55.00
5	-8.87	-10.46	-11.42	-5.71	58.33
Grand Mean of Parents		57.93	Grand Mean of Hybrids		52.93
LSD 5%	2.17	SE	0.87		

4-2-1-2. Silking days (day)

The results of statistical analysis pointed to significant differences within the original and the self-pollinated genotypes (inbred lines and their half diallels) for silking time (Appendix 2).

The first original inbred line (1) was the earliest parental line scoring 54 days till silking (table 13). On the other side, inbreds 4 and 5 were the latest with 59.67 days to silking. Most of the inbred lines showed a rate of inbreeding depression (table 14), however, line 1 showed a high genetic conservative attitude after two generations of selfing and it still ranks the first (56.67 days). Inbred 3 acted in a different way as it silks took longer to stick out (61.67 days).

These variations were transmitted at a different rate to the F1 hybrids in both, the original and self-pollinated populations. The grand mean of the original hybrids (table 13) which was less than their parents (55.67 and 57.87 day, respectively) indicated the earliness of these hybrids for silking. The minimum days (53 day) was sufficient for both original hybrids 1x2 and 1x3 to silk, while 4x5 hybrid was not to silk in a period

less than 60 days. The results showed significant hybrid vigor values for the silking time (table 13). The genes expressed an overdominant effect of the best parent in six original hybrids which exposed negative hybrid vigor percentages reached -2.95% for 2x4 and 2x5 hybrids. The hybrid 1x4 was unique in showing a complete dominance of its earliest parent genes (inbred 1) with zero hybrid vigor.

The variations among self-pollinated inbred lines reflected on their crosses (table 14). Generally, the hybrids have a tendency to silk early based on their grand mean (55.03 days) compared with their parents (59.93 days). The earliest self-pollinated hybrid (1x5) spent 52.67 days to silk, while the hybrid 2x4 required 57.33 days. The inheritance of this trait in the all self-pollinated hybrids was tightly under the overdominant genes of their earliest parent due to the negative hybrid vigor revealed by such crosses. The maximum value was -11.48% for hybrid 3x5, while the minimum value was -2.36% for hybrid 1x2. Abdul-Hamed et al. (2017) and Adebayo et al., 2017 stated similar findings.

Table 13: Means of silking (day) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	54.00	53.00	53.00	54.00	54.33
2	-1.85	56.67	55.67	55.00	55.00
3	-1.85	-1.77	59.33	57.67	59.00
4	0.00	-2.95	-2.80	59.67	60.00
5	0.62	-2.95	-0.56	0.56	59.67
Grand Mean of Parents		57.87	Grand Mean of Hybrids		55.67
LSD 5%	2.21	SE	1.17		

Table 14: Means of silking (day) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	56.67	55.33	54.33	54.00	52.67
2	-2.36	59.67	55.67	57.33	54.00
3	-4.12	-6.71	61.67	56.33	54.00
4	-4.71	-3.92	-7.15	60.67	56.67
5	-7.06	-9.50	-11.48	-6.59	61.00
Grand Mean of Parents		59.93	Grand Mean of Hybrids		55.03
LSD 5%	2.37	SE	0.84		

4-2-1-3. Plant height (cm)

According to the analysis of variance (Appendix 2), all studied genotypes (original and self-pollinated) have significantly differed in plant height. The trait means (table 15) ranged between 166.02 cm and 144.23 cm for the original inbred 2 and 5, respectively.

Selfing has a major effect on maize population as plant height has a sharp decline in the self-pollinated population with a range of 146.95 cm for inbred 4 to 117.26 cm for inbred 1 (table 16). The grand mean of the self-pollinated population (130.71 cm) approved a sort of dwarfness exposed by these inbreds compared with the same mean in their ancestors, the original inbreds (150.55 cm). Compared with their parental inbreds, the original diallel hybrids showed a wider range of plant height (175.67 cm to 145.20 cm for hybrids 1x4 and 4x5, respectively), (table 15).

Half of the original hybrids revealed a positive hybrid vigor (table 15), and 3x4 hybrid achieved its highest percentage (18.18%). The other half diallels showed a negative percentage, but only two of these were significant and 1x2 hybrid gained the lowest (-5.74%). The positive and

the negative hybrid vigor pointed to an over and partial dominant role of highest parent genes, respectively in controlling trait inheritance.

By the same token, the self-pollinated hybrids showed obvious superiority against their best parent (table 16). In view of significant positive hybrid vigor shown by the majority of diallel hybrids, the trait was completely controlled by the genes with an overdominant effect, and the diallel hybrid 1x3 scored the maximum value of such effect (35.24%). These results agreed with those documented by Ali et al., (2017).

Table 15: Means of plant height (cm) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	149.95	156.48	166.55	175.67	168.88
2	-5.74	166.02	163.68	162.30	158.07
3	11.07	-1.41	145.32	174.02	166.00
4	17.15	-2.24	18.18	147.25	145.20
5	12.63	-4.79	14.23	-1.39	144.23
Grand Mean of Parents		150.55	Grand Mean of Hybrids		163.69
LSD 5%	14.54	SE	3.06		

Table 16: Means of plant height (cm) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	117.26	171.20	178.03	173.03	149.77
2	29.08	132.63	161.20	177.83	156.18
3	35.24	21.54	131.65	157.05	161.50
4	17.75	21.02	6.87	146.95	149.62
5	19.75	17.76	22.68	1.81	125.07
Grand Mean of Parents		130.71	Grand Mean of Hybrids		163.54
LSD 5%	22.66	SE	3.04		

4-2-1-4. Ear height (cm)

Significant variations have been detected within both studied populations for ear height trait (Appendix 2). As expected, the performance of some genotypes (inbred 2, 4 and the hybrid 1x4) in this trait was highly affected by plant height trait due to the positive correlation between plant and ear height. Among original inbreds (table 17), line 2 was holding the highest ear (79.08 cm), while the lowest ear height (62.17 cm) was attained by line 3 even after two selfing generations (table 18), but this time hardly reached 48.89 cm.

Of course, the highest value in the self-pollinated population (68.83 cm) acquired by line 4 was less than the highest value of the original population, in a clear reference to the role of inbreeding depression.

This assumption was supported by the grand mean which reached 72.06 cm in the original population, whereas it declined sharply to reach 57.60 cm in the self-pollinated population. Like their ancestors, the original hybrids have a considerable portion of variations. The trait means of these hybrids ranged from 92.72 cm for hybrid 1x4 to 70.78 cm for hybrid 2x4.

The results showed significant hybrid vigor (table 17) ranged between the positive and negative values for ear height trait. However, the inheritance of the trait in five original hybrids was under the effect of over dominance of genes. As 1x4 being one of the previously mentioned hybrids, it showed the largest magnitude of positive vigor (22.83%). The negative hybrid vigor was with the minimal level of -10.50% for hybrid 2x4, indicating the partial dominance of its best parental genes.

In turn, the self-pollinated hybrids clearly varied after two generations of selfing scoring a grand mean of 81.21 which was larger than its original match (79.43), (table 18), hence, their maximum and minimum limits

were at a wider range (68.12 cm to 93.10 cm for 4x5 and 1x4 hybrids, respectively). In view of the significant positive hybrid vigor (table 18), which was mostly revealed by the self-pollinated hybrids (nine hybrids), the inheritance of ear height was guided by the overdominant effect of highest parent genes. The hybrid 1x3 achieved its maximum percentage (60.87%).

Finally, it can be noted that the self-pollination has increasing ear height of hybrids in disagreement with their parents. These outputs partially agreed with previous results (Matin et al., 2017) in view of significant differences and hybrid vigor at both directions in the original hybrids, whereas the self-pollinated hybrids had completely positive values for hybrid vigor.

Table 17: Means of ear height (cm) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	75.48	71.23	84.20	92.72	90.97
2	-9.93	79.08	80.13	70.78	72.93
3	11.55	1.33	62.17	83.06	75.82
4	22.83	-10.50	15.92	71.66	72.50
5	20.51	-7.78	5.40	0.79	71.93
Grand Mean of Parents		72.06	Grand Mean of Hybrids		79.43
LSD 5%	9.69	SE	3.91		

Table 18: Means of ear height (cm) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	55.33	87.45	89.02	93.10	77.00
2	47.74	59.19	84.75	88.45	70.02
3	60.87	43.17	48.89	71.23	82.93
4	35.25	28.50	3.49	68.83	68.12
5	38.16	18.28	48.80	-1.04	55.73
Grand Mean of Parents		57.60	Grand Mean of Hybrids		81.21
LSD 5%	15.23	SE	6.36		

4-2-1-5. Leaf area (cm^2)

Via analysis of variance (Appendix 2), no statistical differences have been detected within the original genotypes (table 19), even so, it was not obstacle against the inheritance of the desirable trait for the superior inbred 2 to its offspring over the subsequent generations. After two rounds of selfing, parental lines have a propensity to increase the range of difference in the trait means (table 20) to exceed the significance threshold (451.54 cm^2 to 363.38 cm^2 for inbreds 5 and 3, respectively). This is not inconsistent with the fact that the self-pollinated parents bring to the light modest performance, which in turn praised the negative response of inbreds to self-pollination in leaf area. These variations were not restricted on inbreds, but they also included their single hybrids. Thus, the self-pollinated inbreds inherited their variations in leaf area to their hybrids (table 20), and the grand mean of diallel hybrids (483.36 cm^2) which was larger than their parents (408.50 cm^2) indicated the superiority of F1 hybrids over their best parents. The trait means in hybrids ranged between 592.26 cm^2 and 408.24 cm^2 for 1x2 and 1x3

hybrids, respectively. The maximum and the minimum values of hybrids indicated that some hybrids responded positively to self-pollination contrary to the others. Overdominant genes were controlling the trait in the all self-pollinated hybrids exposing positive hybrid vigor, whose their top reached 39.32% for hybrid 1x2 (table 20). Whereas, 2x5 hybrid showed partial dominant genes of its earliest parent when it showed the minimum significant negative hybrid vigor (-5.640%). Basically, Al-Falahy (2015) stated similar findings but with higher values.

Table 19: Means of leaf area (cm^2) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	437.73	519.07	530.95	549.18	529.44
2		522.15	552.35	493.75	498.39
3			407.67	505.56	484.53
4				454.94	522.20
5					456.61
Grand Mean of Parents		455.82	Grand Mean of Hybrids		518.54
LSD 5%	ns				

Table 20: Means of leaf area (cm^2) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	397.80	592.26	408.24	507.49	483.83
2	39.32	425.12	497.30	466.43	426.08
3	2.62	16.98	363.38	520.26	496.60
4	25.41	9.72	28.56	404.68	435.15
5	7.15	-5.64	9.98	-3.63	451.54
Grand Mean of Parents		408.50	Grand Mean of Hybrids		483.36
LSD 5%	61.25	SE	4.58		

4-2-1-6. Leaf number (Leaf plant^{-1})

Based on the analysis of variance results, differences were beyond the significance level among genotypes within both the original and self-pollinated populations (Appendix 2). Line 2 from the original population gained the maximum leaves number (14.77), while the minimum (12.20) was the share of line 3 (table 21). After two generations, inbred 2 was still sustaining the highest leaves number (12.46), while inbred 1 acquired the lowest mean (11.06). The grand means of the original (13.52) and self-pollinated populations (11.95) assured that this decline in the general performance is partly due to the direct or indirect effect of methylation status which reinforces the inbreeding depression.

The original hybrids simulated their parents with the significant range of leaves number (15.1 to 13.13 for 1x4 and 2x5 hybrids, respectively), (table 21). The two generations of selfing didn't affect the general order of the hybrids when the hybrid 1x4 was still in the lead of the hybrids for leaf number with a slight increment (0.1) compared with its original counterpart (table 22). This is somewhat similar to the case of hybrid 2x5, but on the opposite direction as it achieved the minimal leaves number (12.67).

The original hybrids directed into significant hybrid vigor for this trait ranged between positive and negative values (table 21). According to the positive hybrid vigor revealed by half of the original hybrids, the transmission of this trait was under the effect of overdominant genes of the best parent. The hybrid 3x5 showed the maximum effect with 11.87% hybrid vigor. This type of gene action has entirely controlled the inheritance of leaf number after two generations of selfing (table 22), as all of the self-pollinated hybrids showed positive hybrid vigor and 1x4

hybrid was in the lead by exposing the highest percentage (23.24%). The other half of original hybrids exposed negative hybrid vigor and the 2x5 hybrid gained the minimum value (-11.06%). These results were in accordance with the recent findings stated by Abdul-Hamed et al. (2017) and Li et al. (2017).

Table 21: Means of leaf number (leaf plant^{-1}) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	14.23	13.30	13.87	15.10	15.00
2	-9.93	14.77	14.00	13.77	13.13
3	-2.58	-5.19	12.20	14.30	14.13
4	6.09	-6.77	3.67	13.79	14.47
5	5.39	-11.06	11.87	4.91	12.63
Grand Mean of Parents		13.52	Grand Mean of Hybrids		14.11
LSD 5%	0.72	SE	2.46		

Table 22: Means of leaf number (leaf plant^{-1}) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	11.06	14.10	14.03	15.20	14.07
2	13.16	12.46	13.60	14.63	12.67
3	20.94	9.15	11.60	12.90	13.47
4	23.24	17.44	4.59	12.33	13.53
5	14.36	1.66	9.49	9.73	12.30
Grand Mean of Parents		11.95	Grand Mean of Hybrids		13.82
LSD 5%	0.65	SE	2.17		

4-2-1-7. Tassel length (cm)

Inbred lines have different ability to produce viable pollens adequate for full seed set formation, which in turn will affect their role as possible pollinators. Hence, tassel length can be a determining factor in the real evaluation of any inbred. The analysis of variance revealed significant differences among genotypes within both populations for tassel length (Appendix 2). Among the original inbreds, inbred 2 showed the tallest tassel with a length of 37.83 cm, while the shortest tassel (30.58 cm) was gained by inbred 1 (table 23).

Inbreeding depression has shortened the tassels in the parental lines after two generations of selfing. The tassel length in the self-pollinated inbreds (table 24) ranged from 35.71 cm for line 3 to 28.27 cm for line 5. This reduction in the tassels length could be easily distinguished via the total means of the original (33.57 cm) and self-pollinated lines (32.01 cm).

Although the superiority of the original lines over their self-pollinated counterparts, however, they were inferior in comparison with their offsprings which revealed a total mean of 36.28 cm.

From the original hybrids, 4x5 gained the highest mean for the trait (40.20 cm) in opposite to hybrid 1x4 that revealed the lowest mean (32.98 cm). The trait means retreated at the next generations (table 24) as the tallest tassel recorded only 37.93 cm for hybrid 2x5, while the shortest was 32.28 cm for hybrid 1x2. At this point, the practicing of self-pollination has decreased the tassel length in the F1 hybrids. This assumption was supported by the grand mean of the trait for the original hybrids (36.28 cm) compared with the self-pollinated hybrids (35.47 cm).

The original hybrids revealed significant hybrid vigor (table 23) ranged between positive and negative estimates (3:2). The highest positive

percentage showed by the hybrid 4x5 (25.17%) indicated the overdominance of its best parent genes (inbred 5) in controlling the trait inheritance. Whereas, partial dominant genes were the key players in the inheritance of the trait in the other hybrids due to their exposing to the negative hybrid vigor, whose there lowest percentage reached -3.59% for hybrid 3x4.

The number of hybrids that significantly showed an overdominant effect of best parental genes increased to five hybrids at the next selfed generations (table 24). At the same time, the maximal range of the positive and negative hybrid vigor (19.96% for 4x5 and -7.48% for 1x2) pointed to a clear depression in the performance of the self-pollinated hybrids in the context of tassel length, which may be attributed to the distinguishable alteration in the genetic and epigenetic performance.

Table 23: Means of tassel length (cm) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	30.58	36.83	35.72	32.98	33.90
2	-2.64	37.83	38.23	36.82	36.50
3	-1.24	1.06	36.17	34.87	36.75
4	5.82	-2.69	-3.59	31.17	40.20
5	5.55	-3.52	1.61	25.17	32.12
Grand Mean of Parents		33.57	Grand Mean of Hybrids		36.28
LSD 5%	2.10	SE	2.74		

Table 24: Means of tassel length (cm) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	31.35	32.28	34.52	33.48	36.95
2	-7.48	34.89	36.73	33.90	37.93
3	-3.35	2.85	35.71	37.10	36.00
4	6.80	-2.85	3.88	29.82	35.77
5	17.86	8.71	0.80	19.96	28.27
Grand Mean of Parents		32.01	Grand Mean of Hybrids		35.47
LSD 5%	2.47	SE	2.82		

4-2-1-8. Tassel branches number (branch tassel^{-1})

A significant deviation has been detected in the performance of both, the original and self-pollinated populations (Appendix 2). Inbreeds still showed a marked decline in the grand performance in response to the selfing process. That was what the grand mean indicated as it reached 14.81 for the original population against 11.53 for the self-pollinated one. From the original parents (table 25), inbred 1 has a tassel with 17.87 branches, while inbred 5 has only 12.47 branches in its tassel. Some of the inbreds responded negatively to the self-pollination in disagreement with the others (table 26). Inbred 1 greatly retreated to give only 8.2 branches, while inbred 5 which had the largest leaf area gave 15.2 branches to become in the lead of the inbreds. The inbreds transmitted the genetic variation to their diallel hybrids in a different norm. The original hybrid 1x4 gave the highest mean (19.8) of the trait (table 25), while the hybrid 2x5 gave the lowest (12.27).

The grand mean of the original hybrids (16.35) proved the superiority of F1 hybrids over their original parents (32.01) for this trait, even so, they

were slightly inferior compared with their descended self-pollinated hybrids (16.48). After all, the self-pollinated hybrids exposed a wider range between 22.57 and 13.07 for 2x3 and 4x5 hybrids, respectively (table 26).

The overdominant genes indicated their control in the inheritance of this trait in the original hybrids based on the positive hybrid vigor, which reached the maximum percentage (28.99%) in the hybrid 4x5. On the other side, the negative hybrid vigor assured the partial dominance of the gene effect and its highest percentage was -29.50% in hybrid 2x5. After two generations of selfing (table 26), the range has widened considerably to be 92.33% in its highest positive limit for hybrid 2x3. Surely, hybrids number which revealed negative hybrid vigor, have been dropped to only two self-pollinated hybrids. Hybrid 4x5 revealed the highest partial dominance effect of its best parent genes (-14.04%).

Table 25: Means of tassel branches number (branch tassel^{-1}) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	17.87	17.60	17.13	19.80	18.80
2	-1.49	17.40	14.87	16.47	12.27
3	-4.10	-14.56	12.53	13.87	14.93
4	10.82	-5.36	0.48	13.80	17.80
5	5.22	-29.50	19.15	28.99	12.47
Grand Mean of Parents		14.81	Grand Mean of Hybrids		16.35
LSD 5%	3.33	SE	5.24		

Table 26: Means of tassel branches number (branch tassel^{-1}) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	8.20	17.87	14.13	17.13	15.40
2	52.27	11.73	22.57	16.87	14.00
3	42.28	92.33	9.93	16.27	17.47
4	35.98	33.86	29.10	12.60	13.07
5	1.32	-7.89	14.91	-14.04	15.20
Grand Mean of Parents		11.53	Grand Mean of Hybrids		16.48
LSD 5%	3.00	SE	9.98		

4-2-1-9. Ears number (ear plant^{-1})

All genotypes significantly varied for this trait according to the analysis of variance (Appendix 2). Among the original inbreds, the highest ear number (1.33) was gained by inbred 2 (table 27), which declined at the next generations to be the most inbreeding-affected inbred with a mean of 1.04 ear per plant, while the performance of inbred 1 was significantly improved to reside in the first spot after two slefing generations (1.29).

Accordingly, it can be told that the inbreds have varied in their response to the self-pollination, but generally, they were still in the same general performance recorded 1.12 ear plant^{-1} for both the original and self-pollinated population. As their parents, the hybrids were significantly varied for this trait. However, the grand mean of ear number in the original hybrids indicated consider a reduction after two generations of selfing. The maximum value in the original hybrids (1.13) was exposed by hybrid 2x3, whereas it was only 1.1 in the self-pollinated hybrids revealed by hybrid 2x5 (table 28).

All the significant hybrid vigor (6 out of 10) was in the undesirable direction in the original hybrids (table 27) as it was negative and reached -25% for both, 1x2 and 2x4 hybrids. This, in turn, indicated the effect of partial dominant genes of their best parent. Selfing has made a significant alteration in the norm of gene action and it scored higher values of hybrid vigor ranged from -3.12% for hybrid 2x3 to -22.58% for hybrids 1x2 and 1x3 (table 28). A significant positive and negative range of hybrid vigor was mentioned by Ali et al., (2017).

Table 27: Means of ears number (ear plant^{-1}) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	1.11	1.00	1.03	1.03	1.10
2	-25.00	1.33	1.13	1.00	1.03
3	-7.24	-15.00	1.00	1.07	1.03
4	-7.24	-25.00	0.34	1.07	1.03
5	-1.26	-22.50	-3.12	-3.12	1.07
Grand Mean of Parents		1.12	Grand Mean of Hybrids		1.05
LSD 5%	0.14	SE	3.19		

Table 28: Means of ears number (ear plant^{-1}) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	1.29	1.00	1.00	1.03	1.07
2	-22.58	1.04	1.03	1.00	1.10
3	-22.58	-3.12	1.07	1.00	1.00
4	-20.00	-9.09	-9.09	1.10	1.00
5	-17.42	0.00	-9.09	-9.09	1.10
Grand Mean of Parents		1.12	Grand Mean of Hybrids		1.02
LSD 5%	0.143	SE	2.52		

4-2-1-10. Kernels rows number (kernel rows ear^{-1})

The analysis of variance revealed significant differences within and between all studied genotypes for kernel rows number (Appendix 2). The highest mean of the trait in the original inbreds (table 29) reached 21.07 for inbred 2, while the lowest (13.07) was exposed by inbred 4.

After two rounds of selfing (table 30), inbreds with maximum and minimum values still apprehended their positions, but with a modest performance. The trait mean noticeably decreased in line 2 to become 19.74, while it rised up in line 4 to become 13.80. The grand means approved that the self-pollination has negatively affected the trait means (16.20 and 15.61 for the original and self-pollinated parents, respectively).

For hybrids, the original set showed a maximum value of 19.13 in hybrid 2x4, while the minimum (14.73) was shown by hybrid 1x3 (table 29). As expected, hybrid means decreased at the next generations of selfing to reach 18.33 for hybrid 2x4 as a maximum value and 13.60 for hybrid 1x5 as a minimum value. The results of statistical analysis (table 30) for kernel rows number cleared that the self-pollination has negatively affected the performance of the hybrids as well as their parents. The original copy of the diallel hybrids showed significantly different hybrid vigor at both directions, positive and negative.

Only two original hybrids inherited the trait by overdominant genes due to their positive hybrid vigor (table 29) and their highest limit (3.52%) was in hybrid 1x4. Eight hybrids revealed negative hybrid vigor indicated the effect of partial dominant genes of their highest parents, and the maximum percentage was -27.22% for hybrid 2x3. The positive hybrid vigor still marked the minority of self-pollinated hybrids (3 hybrids, table

30), while the other hybrids acted in a different way as they showed negative hybrid vigor and the hybrids 1x4 and 1x5 were in the lead of both opposite directions (9.57% and -16.05%, respectively). Findings of some studies (Abdul-Hamed et al., 2017; Li et al., 2017) shared the same findings.

Table 29: Means of kernels rows number (kernel rows ear^{-1}) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	15.13	16.93	14.73	15.67	16.13
2	-19.62	21.07	15.33	19.13	17.47
3	-4.14	-27.22	15.37	15.81	15.93
4	3.52	-9.18	2.88	13.07	15.00
5	-1.49	-17.09	-2.71	-8.41	16.38
Grand Mean of Parents		16.20	Grand Mean of Hybrids		16.21
LSD 5%	2.16	SE	3.20		

Table 30: Means of kernels rows number (kernel rows ear^{-1}) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	13.93	17.00	15.40	15.27	13.60
2	-13.91	19.75	17.93	18.33	17.20
3	7.12	-9.18	14.38	15.60	16.12
4	9.57	-7.16	8.51	13.80	14.27
5	-16.05	-12.90	-0.49	-11.93	16.20
Grand Mean of Parents		15.61	Grand Mean of Hybrids		16.07
LSD 5%	2.15	SE	3.15		

4-2-1-11. Kernels number (kernel row^{-1})

The studied genotypes (original and self-pollinated) differed significantly from each other in kernels number per row (Appendix 2).

Trait means in the original inbreds (table 31) significantly ranged from 35.57 to 29.70 for inbreds 4 and 3, respectively. As inbred 4 was still ranking first after two selfing generations (33), inbred 5 fellback to the bottom and scored only 28.8 kernel row^{-1}. There was a clear evidence for trait mean depression through the grand mean that decreased from 32.72 to 30.73 at the next generations of self-pollination (table 32). An important increase can be detected in more than two kernels per row due to selfing (table 32) to be ranged between 39.03 and 31.93 in the original hybrids (2x5 and 3x5, respectively) and between 41.57 to 34.23 in the self-pollinated hybrids (4x5 and 1x3, respectively). The improved performance of the self-pollinated hybrids was supported by the grand mean of the original and their self-pollinated counterparts that attained 36.57 and 38.08, respectively. The declined or improved performance of the hybrids by the effect of hybridization indicates the fact that hybridization is a highly effective biological process in restructuring of the genetic and epigenetic structure of the organism in question. It may be necessary to mention that only one original hybrid (3x5) was able to exceed the negative significance level (-2.48%).

The superiority of F1 hybrids over their best parents were documented in a significant hybrid vigor form. The overdominance of the parental genes was prevalent in 7 out of 10 (70%) original hybrids marked with positive hybrid vigor (table 31) and the diallel hybrid 2x5 acquired the maximum value (18.04%). The best parent genes were still over dominant in controlling the trait inheritance at the next generations in view of the

positive hybrid vigor that was revealed by all of the self-pollinated hybrids (table 32). The maximum percentage reached a new higher level (31.80%) for hybrid 1x5. Al-Falahy (2015) stated similar results.

Table 31: Means of kernels number (kernel row^{-1}) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	32.54	36.33	37.47	38.07	37.00
2	9.88	33.07	36.47	35.80	39.03
3	15.15	10.28	29.70	38.12	31.93
4	7.03	0.66	7.19	35.57	35.43
5	12.99	18.04	-2.48	-0.37	32.75
Grand Mean of Parents		32.72	Grand Mean of Hybrids		36.57
LSD 5%	4.99	SE	2.16		

Table 32: Means of kernels number (kernel row^{-1}) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	30.22	37.23	34.23	37.13	39.83
2	23.19	29.82	34.43	39.27	37.70
3	7.55	8.18	31.83	41.53	37.91
4	12.53	18.99	25.86	33.00	41.57
5	31.80	26.43	19.11	25.96	28.80
Grand Mean of Parents		30.73	Grand Mean of Hybrids		38.08
LSD 5%	6.34	SE	2.61		

4-2-1-12. 500 Kernels weight (g)

Significant differences have been detected within the original population, whereas the self-pollinated population has no significant differences for 500 kernel weight (table 33). The original inbred line 3 achieved the highest mean (153.07 g). The highest value decreased at the next generations (table 34) to be 130.35g exposed by the self-pollinated inbred 1 in disagreement with the lowest value which noticeably increased to reach 119.01g revealed by inbred 4.

The original inbreds succeeded in transmitting the majority of their genetic variations to the produced hybrids, but the latest was superior over their parents based on the grand mean (152.09 g and 146.04 g respectively). This superiority was also confirmed via the maximum (170.19 g) and the minimum values (129.24 g) gained by 1x3 and 2x5 original hybrids, respectively (table 33). This range was slightly minimized at the next generations to reach 165.63g in its higher limit for hybrid 3x4 (table 34).

The original hybrids revealed significant hybrid vigor ranged between the maximal positive percentage (20.17%) and negative percentage (-2.91%) attained by 1x4 and 2x5 hybrids, respectively (table 33). The self-pollinated hybrids enthused to a different level of gene expression when the trait mean was entirely guided by the overdominant type of gene action in view of the positive hybrid vigor detected in all hybrids with a maximum value of 31.53% for hybrid 3x4 (table 34).

The positive and the negative hybrid vigor represent the crucial role of the over and partial dominant effect of parental genes in controlling the trait inheritance, respectively. Abdul-Hamed et al. **(**2017) and Bisen et al., (2017) pointed to similar results.

Table 33: Means of 500 kernels weight (g) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	133.22	145.69	170.19	166.31	148.27
2	9.36	133.11	157.62	142.14	129.24
3	11.19	2.97	153.07	163.98	160.24
4	20.17	2.71	7.13	138.39	137.26
5	11.30	-2.91	4.68	-0.82	116.03
Grand Mean of Parents		134.76	Grand Mean of Hybrids		152.09
LSD 5%	17.37	SE	2.14		

Table 34: Means of 500 kernels weight (g) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	130.35	139.36	153.71	150.43	145.18
2	6.91	128.78	143.54	129.60	150.56
3	17.92	11.46	125.93	165.63	149.19
4	15.41	0.64	31.53	119.01	133.19
5	11.38	16.91	18.47	10.58	120.45
Grand Mean of Parents		124.90	Grand Mean of Hybrids		146.04
LSD 5%	20.46	SE	2.60		

4-2-1-13. Plant yield (g)

The original and the descended self-pollinated populations revealed significant differences according to the results of the analysis of variance (Appendix 2). A wide range of grain yield trait was shown by the original inbreds (176.26 g to 94.22 g for inbred 2 and 5, respectively), (table 35). Inbred 2 which gained the highest mean for most traits including leaf number, ear number and row number was still in the lead after two rounds

of selfing, however, there was an obvious effect of inbreeding as it achieved only 101.80 g (table 36). The self-pollinated inbred 1 showed a higher rate of depression when its performance declined to establish the minimum grain yield (62.43 g).

The previously detailed results could be summarized via the huge gap in the grand mean between the original (118.18 g) and the self-pollinated inbreds (79.38 g) which pointed to the negative effect of the selfing and the growing role of DNA methylation along with self-pollination.

The original hybrid 1x5 revealed the highest yield per plant reached 163.92 g for hybrid 1x5, while the lowest was 3x5 revealed 126.75 g (table 35). The grand mean of the original hybrids (149.59 g) was higher than their parents, nevertheless it was lower than its match in the self-pollinated population (138.30 g), which confirmed the retreated performance of the hybrids under the effect of self-pollination. Their values also retreated to reach 162.05 for hybrid 1x4 as the highest and 118.43 g for hybrid 4x5 as the lowest (table 36). The high yield of hybrid 1x4 has not accidentally occurred but as a consequence of their parents superiority in most of the traits.

All the original hybrids were with significant hybrid vigor (table 35), four of these exposed negative percentages ranged from -12.11% for hybrid 2x3 to -18.90% for hybrid 2x4 and demonstrated the trait subordination to the partial dominance of the parental genes. The majority of hybrids revealed positive hybrid vigor ranged between 19.54% for hybrid 3x5 to 49.71% for hybrid 1x5 as illustrated the effect of over dominant genes which were fully in charged at the next selfing generations (table 36) with percentages of 103.25% for hybrid 1x4 and 23.61% for hybrid 2x5. These

findings agreed with those stated by Ali et al., (2017), Li et al., (2017) and Matin et al., 2017.

Table 35: Means of plant yield (g) for original inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	109.49	146.34	156.78	163.63	163.92
2	-16.97	176.26	154.92	142.95	152.79
3	43.19	-12.11	106.04	157.20	126.75
4	49.44	-18.90	48.25	104.91	130.59
5	49.71	-13.32	19.54	24.48	94.22
Grand Mean of Parents		118.18	Grand Mean of Hybrids		149.59
LSD 5%	18.05	SE	9.46		

Table 36: Means of plant yield (g) for self-pollinated inbred lines (diagonal values) and their half diallel hybrids (above diagonal values), and hybrid vigor (below diagonal values) in maize.

Parents	1	2	3	4	5
1	62.43	144.09	133.15	162.05	122.33
2	41.54	101.80	139.50	137.34	125.83
3	68.92	37.03	78.83	161.95	138.37
4	103.25	34.91	103.13	79.73	118.43
5	65.01	23.61	75.54	48.55	74.14
Grand Mean of Parents		79.38	Grand Mean of Hybrids		138.30
LSD 5%	25.27	SE	8.84		

4-2-2. Combining ability

4-2-2-1. Anthesis days

The result of the genetic analysis (Appendix 2) revealed that the mean squares of the both the general (GCA) and specific combining ability (SCA) have significantly varied for anthesis time, which in turn pointed to the importance of both the additive and non-additive gene action in the inheritance of this trait in the original and self-pollinated inbreds. Nevertheless, the non-additive gene action was more effective based on the σ^2gca/σ^2sca ratio which was less than unity (0.421) and it was minimized to become 0.014 at the next generations. Furthermore, the degree of dominance (1.54 and 8.43, respectively) confirmed the recent findings (table 62). The broad sense heritability was high and close in both, original and selfing generations (94.16 and 94.42 respectively), while the narrow sense heritability decreased from 43.05 to 2.58 across those generations. Similar findings were observed by Bawa et al. (2017).

The results of the general ($\hat{g}_i$) and specific ($\hat{S}_{ij}$) combining ability estimates showed that the original inbreds 1 and 2 have revealed negative general effect in the inheritance of anthesis trait (table 37), consequently, all their hybrids tasseled in a less duration compared with the grand mean. Inbred 1 was in the lead with the highest negative values of GCA estimates (-1.286), while inbred 5 have differed in its combining behavior as it exposed the highest positive GCA value (1.048). Such inbreds had undesired effect when it inherited late anthesis to their hybrids.

After two rounds of self-pollination (table 38), the additive gene action of inbred 5 negatively affected the trait mean, hence the inbred joined the inbreds marked with negative GCA effect (inbred 1 and 2). Inbred 1 still

holding the highest negative value (-0.610), suggesting a greater ability for such inbreds to confer early anthesis to their pedigree. In addition, inbreds 3 and 4 still showed an undesirable performance regarding the positive GCA effect and inbred 4 was in the lead (0.629).

The ability of an inbred to inherit earliness of anthesis to a specific hybrid compared with the general performance of this inbred (SCA) was significantly negative in nearly half of the original hybrids (6 out of 10). The hybrid 1x3 gained the highest SCA negative effect (-2.016), while the hybrid 4x5 achieved the highest positive SCA (0.603).

The self-pollinated hybrid 1x2 was derived from parents with negative GCA effects, yet, it showed the only non-significant positive SCA (0.429). Among the eight hybrids which revealed negative SCA, hybrid 3x5 was the highest (-3.00) indicated the ability of inbred 5 to pass down the early anthesis to its hybrid in spite of its different performance at two selfing generations ago.

The variance of the additive ($\sigma^2 gii$) and non-additive ($\sigma^2 Sii$) gene action was positive in the original inbreds (table 37). After two generations of selfing, one value of negative variance for GCA can be noticed (-0.041) in inbred 2, whereas it reached 0.347 in inbred 4 as the highest positive value (table 38). The variance of SCA was still completely positive, where the highest (5.537) was shown by inbred 5 and the lowest (0.616) was detained by inbred 4. Inbred 5 performance was exceptional when it exposed the lowest value (0.083) at the original generation, however, it revealed the highest $\sigma^2 Sii$ at the next generations of selfing.

Table 37: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for anthesis in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-1.286	-0.635	-2.016	-0.730	-0.206	1.633	0.704
2		-0.857	-0.444	-1.825	-1.635	0.715	1.225
3			0.524	-0.206	0.317	0.254	0.492
4				0.571	0.603	0.306	0.447
5					1.048	1.077	0.083
S.E.	gi =0.164		sii =0.423				

Table 38: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for anthesis in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-0.610	0.429	-1.619	-2.286	-2.429	0.323	2.308
2		-0.086	-2.143	-1.143	-2.952	-0.041	2.598
3			0.295	-1.524	-3.000	0.039	3.844
4				0.629	0.000	0.347	0.616
5					-0.229	0.004	5.537
S.E.	gi =0.253		sii =0.653				

4-2-2-2. Silking days

Based on the results of the genetic analysis for the silking trait (Appendix 2), mean squares of both GCA and SCA have significantly varied which indicated equal importance for the both additive and non-additive action in controlling the trait inheritance. This conclusion was assured by σ^2gca/σ^2sca ratio and **ā** (table 62) which were so close to the unity (0.966 and 1.02, respectively). Selfing has played a pivotal role in the genetic action and the non-additive type was more prevalent in transmitting the silking time in the self-pollinated inbreds based on σ^2gca/σ^2sca ratio (table 62) which was far away from the unity (0.071) in addition to the overstated **ā** value (3.75). The broad sense heritability for the original set (94.42%) was higher than the narrow sense heritability (60.23%), however the different between them was greatly wider in response to

selfing (93.60 and 11.64, respectively). Al-Naggar et al. (2017) disagree with these results as they assured the presence of different gene action which guided the trait inheritance.

The GCA effect of each inbred and SCA effect of each single hybrid in the original population for the silking trait were listed in table 39. It can be noticed that inbred 1 was the best combiner for silking time in both, the original and self-pollinated versions (-2.295 and -1.476, respectively). Meanwhile, the original inbred 5 took a reverse direction by recording the maximum positive GCA effect (1.324).

Five original hybrids exposed negative SCA effects, hybrid 1x3 achieved the maximum value (-1.905), whereas, hybrid 4x5 acquired the highest positive SCA (1.190). Self-pollination was effective enough to increase the hybrids number marked with negative SCA effects (table 40), where seven hybrids revealed negative specific combining effects to reach the maximum (-3.095) in the hybrid 3x5.

Table (40) cleared that the inbreds exposed totally positive variance of effect for GCA and negative for SCA. These findings can be summarized by the fact that parents generally have been more stable in their inheritance pattern compared with specific hybrids combination which was more volatile.

Table 39: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for silking in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-2.295	-0.190	-1.905	-1.190	-1.095	5.218	-0.340
2		-0.914	-0.619	-1.571	-1.810	0.786	-0.379
3			0.800	-0.619	0.476	0.590	-0.893
4				1.086	1.190	1.129	-0.538
5					1.324	1.702	-0.395
S.E.	gi =0.258		sii =0.667				

Table 40: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for silking in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-1.476	-0.095	-1.381	-2.000	-2.429	2.122	1.140
2		0.238	-1.762	-0.381	-2.810	-0.001	0.920
3			0.524	-1.667	-3.095	0.217	2.992
4				0.810	-0.714	0.598	-0.320
5					-0.095	-0.048	5.163
S.E.	gi =0.277		sii =0.715				

4-2-2-3. Plant height

The additive gene action (GCA) did not exceed the significance threshold in both the original and self-pollinated inbreds (Appendix 2) in disagreement with SCA effects whose its significancy was assured via quite low σ^2gca/σ^2sca ratio (0.023). The significant effect of the non-additive action was consistently transmitted to the next generations, as the σ^2gca/σ^2sca ratio (0.026) was almost the same compared with its value in the original copy (table 62). Selfing had a positive effect on both heritability senses, however the broad one was more responsive as it increased from 82.94 to 87.14, whereas the narrow sense heritability was still too low even after two selfed generations (3.67 to 4.28) as a result of the low additive effect. Kamara, (2016) stated similar results confirmed the same type of gene action.

Five out of the ten original hybrids revealed significant positive SCA effects (table 41) and the highest (15.291) indicated the ability of inbred 1 to produce taller plants in hybrid 1x4 regardless of its partner (inbred 4) that has non-significant GCA effect. The other two original hybrids (1x2 and 4x5) were with significant SCA effects, the highest value (-9.337) was gained by hybrid 4x5.

Likewise the original inbreds, the genetic effect in the self-pollinated inbreds was classified as a non-additive type in line with the SCA data (table 42). Most of the self-pollinated hybrids have positive SCA effects and hybrid 1x3 exposed the maximum effect (25.941). Other hybrids such as 3x4 tended to produce shorter plants based on the negative SCA effect (-1.453). It seems that inbred 1 assumed its dominance in the inheritance of this trait again as its hybrid response to the self-pollination.

Original inbred 5 was the most variegated in view of $\sigma^2 gii$ values (table 41), which showed the direction of the inbred to inherit the plant height in a different pattern. Inbred 2 was distinct and played the same role but in the opposite direction when it gave the highest negative value but for $\sigma^2 Sii$. This was inconsistent with the performance of inbred 4 that acquired the maximum value (73.439).

Inbred 5 conserved its exceptional attitude as it gained the highest positive value (42.54) of $\sigma^2 gii$ at the next selfed generations. The $\sigma^2 Sii$ values have magnified to reach 174.000 in inbred 1 compared with inbred 5 which revealed the maximum negative value (-147.204).

Table 41: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for plant height in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	1.662	-6.875	4.860	15.291	12.089	0.603	45.286
2		2.388	1.267	1.199	0.546	3.544	-88.130
3			0.720	14.590	10.148	-1.641	8.688
4				-0.595	-9.337	-1.806	73.439
5					-4.176	15.278	7.200
S.E.	gi =ns		sii =4.382				

Table 42: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for plant height in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-1.290	17.593	25.941	16.604	5.371	-3.582	174.000
2		2.298	5.520	17.816	8.200	0.036	-13.458
3			0.784	-1.453	15.031	-4.631	55.470
4				5.121	-1.189	20.974	-56.125
5					-6.913	42.540	-147.204
S.E.	gi =ns		sii =6.829				

4-2-2-4. Ear height

The results of the genetic analysis (Appendix 2) proved the significant differences of the general and specific combining ability effects exclusively in the original inbreds, which in turn described the importance of both types, the additive and non-additive gene action in the inheritance of ear height. However, the additive effect has been recognized as the most important type of gene action guiding the inheritance of this trait based on the σ^2gca/σ^2sca ratio which was enormously less than the unity in both the original and self-pollinated populations (0.054 and 0.021, respectively). By the same token, the dominance degree (4.31 and 6.83 respectively), toughen the recent conclusions. Therefore, at a time as the value of broad sense heritability was rising (86.75% to 89.48%), the value of the narrow sense heritability decreased due to selfing (8.42% to 3.68%). One past review (Wolde et al., 2017) disagreed with these findings.

Through the results of GCA effects in the original population (table 62), it can be observed that inbreds 1 and 4 contributed in the augmentation of the ear height in their crosses in view of their positive GCA effects whose their maximum value (4.031) gained by inbred 1. In the opposite direction, inbreds 2, 3 and 5 resulted in the low ear of their hybrids due to

their negative GCA effects in which inbred 3 scored the highest negative values (-2.046). The studied parental inbreds revealed a non-significant GCA after performing the self-pollination. The ability of inbred to inherit the ear height into specific hybrid compared with the general performance of the same inbred in all of its crosses (SCA) was positive in six hybrids (1x3, 1x4, 1x5, 2x3, 3x4 and 3x5) in the original population (table 43), and the superiority of hybrid 3x4 was documented (11.635). The significant negative SCA effects reached their maximum level in the hybrid 1x2 (-8.544).

Self-pollination has impacted the specific ability of the inbreds to combine (SCA) as seven hybrids were detected with significant positive SCA (table 43) and hybrid 3x5 acquired the highest effect (15.995). The other three hybrids revealed negative SCA effects, but didn't exceed the significance level.

Table (44) could provide fresh insights into inbred 1 with its highest positive variance for both GCA and SCA effects (15.288 and 70.758, respectively), while the highest negative values reached -0.954 in inbred 4 and -1.056 in inbred 3. After two generations of the self-pollination, inbred 5 seemed with highest positive variance for GCA (16.601) and inbred 3 for SCA (99.584), whereas, inbred 2 and 5 were with negative values as reached -0.704 and -15.421 for GCA and SCA, respectively (table 44).

Table 43: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for ear height in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	4.031	-8.544	5.237	11.635	10.784	15.288	70.758
2		-1.231	6.432	-5.036	-1.987	0.557	1.285
3			-2.046	8.059	1.711	3.227	-1.056
4				0.073	-3.724	-0.954	33.238
5					-0.826	-0.276	-0.930
S.E.	gi =1.131		sii =2.919				

Table 44: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for ear height in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	2.459	10.364	15.264	14.655	5.560	3.677	80.217
2		1.290	12.166	11.173	-0.255	-0.704	11.636
3			-2.043	-2.710	15.995	1.805	99.584
4				2.649	-3.514	4.650	4.627
5					-4.355	16.601	-15.421
S.E.	gi =ns		sii =4.589				

4-2-2-5. Leaf area

The combining ability was not significant in its general and specific approaches for leaf area in the original population (Appendix 2), while the continuation of the selfing process resulted in a significant deviation in the SCA effect. The σ^2gca/σ^2sca ratio (0.006) pointed to an unambiguous presence of the non-additive gene action in controlling this trait in the self-pollinated inbreds (table 62). Accordingly, the broad sense heritability reached 90.40%, while the narrow sense heritability dropped to 1.15%. These findings were supported by a previous study (Hussein et al. 2015) aimed to significant role for the non-additive gene action in

controlling the trait. Six hybrids (1x2, 1x4, 1x5, 2x3, 3x4 and 3x5) exposed positive SCA effects (table 45), where hybrid 1x2 was in the lead of order with the highest value (116.882), whereas hybrid 2x5 gained the maximum value in the opposite direction (-43.211).

The variance of SCA effect was totally positive (table 45), and the first self-pollinated inbred (1) was the most varied in the inheritance of its positive effect to its diallel hybrid (4086.525) compared with the weaker performance which was revealed by the fifth inbred (5) as it showed the lowest $\sigma^2 Sii$ value (2.797).

Table 45: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for leaf area in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	5.280	116.882	-40.983	45.485	20.960	-10.451	4086.525
2		11.692	41.674	-1.991	-43.211	98.370	3893.238
3			-14.473	78.006	53.481	171.131	2257.332
4				-1.682	-20.764	-35.498	999.787
5					-0.817	-37.661	2.797
S.E.	gi = ns		sii =18.458				

4-2-2-6. Leaf number

The results of the genetic analysis (Appendix 2) showed significant differences in the effect of GCA and SCA for leaf number. The σ^2gca/σ^2sca ratio (0.115), (table 62) describes the gene action underlying plant height by which the non-additive gene action was more dominant in the inheritance of this trait. The broad sense heritability was greatly more than the narrow sense heritability and it responded to selfing in a better way as it has increased from 92.66% to 97.19% in disagreement with the narrow sense which has largely decreased from 17.37% to 3.52%. Dar et al. (2017) documented different results, as the additive gene action was the most important.

The highest positive GCA effect in the original inbreds (table 46) was exposed by inbred 1 (0.323), while inbreds 3 and 5 were with negative GCA effects (-0.397 and -0.211, respectively). The positive and negative genetic performance of these inbreds did not change after two generations of selfing, however their values have distorted. Inbred 4 revealed the highest positive GCA effects reached 0.250 (table 47). The non-additive gene action has become more pronounced at this generation based on the σ^2gca/σ^2sca ratio (0.019). Hybrid 1x5 was the highest and scored 0.976. Only three original hybrids (1x2, 2x4 and 2x5) accounted to show negative SCA effects and the highest (-0.972) was gained by hybrid 1x2.

At the next generations (table 47), self-pollination has boosted the specific ability of inbreds to inherit the desirable trait, hence eight hybrids revealed positive SCA effects and hybrid 1x4 acquired the highest (1.704) against hybrids 2x5 that was with the most negative SCA effect (-0.514).

Most of the original inbreds have a variable ability to inherit their positive effect of GCA (table 46) to reach the highest (0.152), however, their SCA variance was completely positive. At the next generations (table 47), inbred 1 appeared with a negative GCA variance in opposite of the other inbreds included inbred 3 which was with the highest positive value (0.076). The SCA variance was also positive in all inbreds and ranged between 0.342 in inbred 5 to 1.625 in inbred 1.

Table 46: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for leaf number in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	0.323	-0.972	0.028	0.617	0.976	0.099	0.502
2		0.037	0.447	-0.431	-0.605	-0.004	0.309
3			-0.397	0.533	0.828	0.152	0.134
4				0.248	0.517	0.056	0.116
5					-0.211	0.039	0.500
S.E.	gi =0.084		sii =0.217				

Table 47: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for leaf number in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	0.049	0.749	1.070	1.704	0.942	-0.002	1.625
2		0.105	0.580	1.081	-0.514	0.007	0.570
3			-0.282	-0.265	0.673	0.076	0.461
4				0.250	0.208	0.058	1.188
5					-0.122	0.011	0.342
S.E.	gi =0.075		sii =0.195				

4-2-2-7. Tassel length

According to the genetic analysis results (Appendix 2), all genotypes (original and self-pollinated) have differed regarding SCA and GCA effects beyond the significance level for tassel length trait.

The additive and non-additive gene actions were both participated in the genetic expression of the trait (table 62), however, the non-additive gene action was more prevalent according to the σ^2gca/σ^2sca ratio (0.272) and this kind of gene action was noticed to be more effective after two generations in view of σ^2gca/σ^2sca ratio (0.105). The broad sense heritability has not been considerably changed by the effect of selfing, where, it reached 93.90 and 92.66 respectively, while the narrow sense

heritability has negatively responded to selfing as it almost halved (33.07 to 16.11). Alfalahi et al. (2012) stated a different gene action.

The genetic performance of inbreds compared to the GCA effects, were fairly consistent across selfed generations no matter the different values. The GCA effect ranged in the original inbreds between 1.683 and -1.667 in inbreds 2 and 1, respectively (table 48). Self-pollinated inbred 3 had the highest value (1.413), (table 49), while inbred 4 had the most negative GCA effect (-0.857).

Seven hybrids from the original population revealed positive SCA effects (table 48), the highest value (5.643) was observed in hybrid 4x5, meanwhile, the maximum negative value (-0.593) was shown by hybrid 3x4. The same proportion was observed after two generations of self-pollination, but this time hybrid 1x5 was at the top (3.872) against hybrid 1x2, the owner of the most negative specific effect (-1.859).

Inbred 5 from the original population lonely revealed the negative variance for GCA reached -0.035 (table 48), while the rest original inbreds directed to be more fluctuated in transmitting the increment in the trait mean. The SCA variance had a wider range (8.679 for inbred 5 and -1.390 for inbred 3). The self-pollinated inbreds varied positively in their GCA effect. Inbred 4 performance at the top (1.935) was unlike that of inbred 5, which had very modest performance (0.086). Inbred 3 lonely exposed the negative variance for SCA (-1.149), while the value 8.222 showed by inbred 5 has been recorded as the highest value (table 49).

Table 48: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for tassel length in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-1.667	1.439	1.201	-0.005	0.287	2.734	-0.982
2		1.683	0.368	0.479	-0.463	2.788	-1.298
3			0.805	-0.593	0.665	0.602	-1.390
4				-0.723	5.643	0.478	8.627
5					-0.098	-0.035	8.679
S.E.	gi =0.245		sii =0.632				

Table 49: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for tassel length in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-0.850	-1.859	-0.360	0.876	3.872	0.660	3.419
2		0.679	0.324	-0.236	3.326	0.399	1.865
3			1.413	2.230	0.659	1.935	-1.149
4				-0.857	2.695	0.672	1.325
5					-0.385	0.086	8.222
S.E.	gi =0.288		sii =0.744				

4-2-2-8. Tassel branches number

Both the original and self-pollinated inbreds revealed significant additive and non-additive gene effects (Appendix 2). Nevertheless, the genetic action was classified as a non-additive type in line with the data of the combining ability. Also, the σ^2gca/σ^2sca ratio in both the original and self-pollinated populations (0.494 and 0.021, respectively) was a clear evidence for trait mean trending to be more controlled by non-additive gene action along with the selfing generations (table 62). The headed value of heritability from 79.50% to 93.55% in its broad sense and the retreated from 39.52% to 3.85% in its narrow sense in response to self-population confirmed the previous findings which differed with what was obtained by Alfalahi et al., (2012). The highest GCA effect (2.004) apprehended by original inbred 1 (table 50) indicated its efficiency to

combine positively via its crosses compared with the grand mean, while inbred 3 acted completely in a different way by detaining the most negative GCA effect (-1.310).

The GCA effects were inherited in a significant range to the next generations (table 51) and inbreds 2 and 1 were at the ends of the comparison (0.828 and -1.149, respectively). The majority of the original hybrids (8/10) revealed positive SCA effects (table 50) and ranged between the highest positive value (6.721) attained by hybrid 2x3 and the lowest negative value (-1.894) gained by hybrid 4x5.

The general attitude was almost the same with respect to $\sigma^2 gii$ values before and after two rounds of selfing, meanwhile $\sigma^2 Sii$ values indicated major alterations in the inbreds genetic recital. This can be seen clearly through the $\sigma^2 Sii$ values that were negative in most cases (table 50), yet completely reversed to be positive after two rounds of selfing (table 51).

Table 50: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for tassel branches number in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	2.004	-0.381	0.600	1.886	1.857	3.902	-2.995
2		0.137	0.200	0.419	-2.810	-0.094	-2.747
3			-1.310	-0.733	1.305	1.604	-4.618
4				0.070	2.790	-0.108	-1.479
5					-0.901	0.699	1.446
S.E.	gi =0.388		sii =1.003				

Table 51: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for tassel branches number in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-1.149	3.359	0.263	3.516	1.525	1.227	4.199
2		0.828	6.721	1.273	-1.851	0.593	16.017
3			0.190	1.311	2.254	-0.056	12.864
4				-0.063	-1.894	-0.088	1.948
5					0.194	-0.054	0.325
S.E.	gi =0.351		sii =0.905				

4-2-2-9. Ears number

According to the genetic analysis (Appendix 2), the mean squares of both the general (GCA) and specific combining ability (SCA) ranged between the significance and non-significant effect for ear number depending on the inbreds response to self-pollination. The results indicated the importance of both the additive and non-additive gene action in the transmission of this trait, but the largest proportion was for the non-additive gene action based on σ^2gca/σ^2sca ratio (table 62) which was greatly less than the unity (0.167). However, it has diminished to 0.152 at the next selfed generations. These findings have been assured via the dominance degree which increased from 2.44 to 2.57 across these generations. The heritability has noticeably decreased in both senses after performing the selfing, where the broad heritability reached 70.39% and 63.30%, whereas the narrow heritability reached 17.65% and 14.73%, respectively. Konate et al. (2017) stated a different gene action in controlling this trait.

The general ability of the original inbreds to combine has differed significantly in disagreement with the self-pollinated inbreds (table 52, 53).

Among the five original inbreds, inbred 2 lonely marked with the positive GCA effect (0.059), meanwhile inbreds 3 and 4 exposed the same negative GCA effect (-0.021). This type of gene action is further altered in response to selfing in disagreement with the SCA effects. The original inbreds contained the highest positive SCA effect (0.046) which was for hybrid 1x5, whereas the highest negative SCA effect (-0.125) was gained by hybrid 1x2. These effects have decreased after selfing to range 0.057 to -0.088 in hybrids 2x5 and 1x2, respectively (table 53).

The effect variance (table 52) showed that most of the original inbreds were with 0.000 for the variance of GCA effects except inbred 2 (0.003). These values consistently transmitted to the next generations not including the highest value that decreased to 0.002 in inbred 1 (table 53). The effect variance for SCA showed that the inbreds were generally with negative values to reach the maximum of -0.009 in inbred 3, but inbred 2 was unique in revealing the positive value (0.002). Some of these inbreds showed enough genetic stability not to change after re-selfing, like inbred 1 which has been marked with the highest negative variance of effect, while the lowest (-0.008) was observed in line 4.

Table 52: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for ears number in the original maize population.

Parents	1	2	3	4	5	σ^2gii	σ^2Sii
1	-0.004	-0.125	-0.012	-0.012	0.046	0.000	-0.004
2		0.059	0.026	-0.108	-0.083	0.003	0.002
3			-0.021	0.043	-0.003	0.000	-0.009
4				-0.021	-0.003	0.000	-0.005
5					-0.012	0.000	-0.007
S.E.	gi =0.016		sii =0.042				

Table 53: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for ears number in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	0.050	-0.088	-0.082	-0.058	-0.044	0.002	-0.004
2		-0.018	0.019	-0.023	0.057	0.000	-0.006
3			-0.024	-0.018	-0.037	0.000	-0.007
4				-0.014	-0.046	0.000	-0.008
5					0.005	0.000	-0.007
S.E.	gi =ns		sii =0.043				

4-2-2-10. Kernels rows number

Via the results of the genetic analysis (Appendix 2), GCA effects were significant across generations for kernels rows per ear. Meanwhile, SCA effects approved to be significant just at the original population.

The additive and non-additive genes equally participated in the inheritance of the trait mean in the original population based on the σ^2gca/σ^2sca ratio which reached 1.161 in addition to the **ā** value which was virtually one (0.93). The additive gene action has become more influential after two selfing generations as σ^2gca/σ^2sca ratio and **ā** value reached 5.146 and 0.225, respectively (table 62). Although different values of broad (84.59%) and the narrow sense heritabilities (59.13%) have been detected in the original population, however they both tended to be comparable after two generations of selfing (82.17% and 74.92%, respectively). Anilkumar et al., (2017) reached similar results.

The original inbreds revealed significant positive and negative GCA effects ranged from 1.962 in inbred 2 to -0.788 in inbred 4 (table 54). At the next two generations, only inbred 2 revealed positive GCA effects reached 2.064 (table 55), while the other revealed negative GCA effects and inbred 1 was in the lead (-0.911).

The performance of the produced hybrids varies and greatly depended on the extent to which the parental genes are compatible. Such issue was assured via different performance exposed by hybrids that have a common parent like 2x3 and 2x4, which showed the most divergent values for SCA effects (-2.167 and 1.749, respectively).

The variance of GCA and SCA effects (table 55) was in the same direction across the selfing generations. Inbreds directed to combine positively in general and negatively in specific. Only the second inbred (2) was performing exceptionally when it trended to combine negatively at the next generations.

Table 54: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for kernels rows number in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-0.504	-0.735	-0.300	0.749	0.424	0.207	-1.853
2		1.962	-2.167	1.749	-0.710	3.803	0.622
3			-0.673	1.064	0.392	0.405	-0.287
4				-0.788	-0.426	0.574	-0.666
5					0.003	-0.048	-1.971
S.E.	gi =0.252		sii =0.650				

Table 55: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for kernels rows number in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-0.911	-0.072	0.636	0.894	-1.133	0.783	-1.474
2		2.064	0.194	0.986	-0.508	4.213	-1.881
3			-0.243	0.560	0.720	0.012	-1.880
4				-0.635	-0.742	0.356	-1.426
5					-0.275	0.028	-1.435
S.E.	gi =0.251		sii =ns				

4-2-2-11. Kernels number

The results of the genetic analysis (Appendix 2) showed that the inbreds revealed non-significant GCA effects in the original and the self-pollinated populations in disagreement with the SCA effects. The σ^2gca/σ^2sca ratio in the original and self-pollinated inbreds (0.042 and 0.016) proved the solitary of genes with the non-additive action in the inheritance of this trait (table 62). The broad sense heritability has positively heightened by selfing (65.90% and 78.89%), while the narrow sense heritability has declined from 5.11% to 2.44% in the original and self-pollinated populations, respectively. These findings were in the opposite direction with what were obtained by Dar et al. (2017).

Half of the original hybrids (5/10) exposed significant positive SCA effects (table 56). The original hybrid 2x5 owned the highest value (3.857), while the only significant negative SCA effects (-1.762) were distinct of the hybrid 3x5.

The SCA effects were also significant at the next generations (table 57) with obvious increment in their values. Therefore, the transmission of the trait was strictly reliable on the non-additive gene action based on σ^2gca/σ^2sca ratio (0.016). The significant positive SCA effects were observed in seven hybrids and the value of 4.788 in the hybrid 1x5 was stated as the maximum (table 57). Inbred 5 has entirely shifted its performance as it combined specifically in all of its crosses to raise up the rows number, at the same time no negative SCA effects has been described to be significant.

The $\sigma^2 Sii$ values were negative in all original (table 56) and self-pollinated inbreds (table 57), which in turn confirmed the undesirable direction of the inbreds in reducing the trait mean from one hybrid to another.

Table 56: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for kernels number in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	0.318	0.436	3.051	1.485	1.799	-0.153	-7.405
2		0.294	2.076	-0.757	3.857	-0.168	-5.735
3			-1.188	3.048	-1.762	1.156	-3.715
4				0.978	-0.427	0.702	-8.301
5					-0.402	-0.093	-5.252
S.E.	gi =ns		sii =1.505				

Table 57: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for kernels number in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-0.704	3.093	-0.407	0.532	4.788	0.085	-9.000
2		-0.791	-0.120	2.752	2.741	0.214	-11.756
3			-0.291	4.519	2.454	-0.327	-11.105
4				1.670	4.147	2.379	-4.821
5					0.115	-0.398	-2.096
S.E.	gi =ns		sii =1.911				

4-2-2-12. 500 kernels weight

The results of the genetic analysis (Appendix 2) indicated significant effects of the additive (GCA) and non-additive gene action (SCA) in the original inbreds. However, the significancy was exclusive to the non-additive gene action in the self-pollinated population for 500 kernel weight.

The σ^2gca/σ^2sca ratio was minimized from 0.490 to 0.011 due to selfing indicating the trend of this trait to be inherited mainly via the non-additive gene action during the repetition of the self-pollination that was supported by the dominance degree range which magnified from 1.43 to 9.68 (table 62). In line with the above, the broad sense heritability (86.01%) was about the double of the narrow sense heritability (42.58%),

which mostly retreated (1.64%) after two selfing generations compared with the broad sense heritability (78.74%). Matin et al. (2017) pointed to comparable results.

The GCA effects ranged in the original inbreds from 11.465 for inbred 3 to -10.119 for inbred 5 (table 58). At the same time, the ability of these inbreds to combine specifically ranged between 16.052 in hybrid 1x4 to -1.677 in hybrid 1x5. The range was widened considerably due to the self-pollination to reach the maximum (24.798) in hybrid 3x4 (table 59), in the opposite of the hybrid 2x4 that was in the other direction with the lowest negative value (-5.044).

Both the additive and non-additive types of gene action varied from one inbred to another (table 59). The highest positive variance of GCA effect ranged 128.371 in inbred 3 to -1.5844 (negative value) which has been lonely revealed by inbred 4. The effect variance of SCA in the original inbreds was completely negative and ranged -2.056 in inbred 1 to -138.793 in inbred 2. However, this effect has been improved by the impact of the selfing to reach 51.641 as a maximum in line 4 and -127.234 as a minimum in inbred 1.

Table 58: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for 500 kernels weight in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	2.714	1.943	9.694	16.052	9.361	4.287	-2.056
2		-5.284	5.122	-0.112	-1.677	24.843	-138.793
3			11.465	4.971	12.573	128.371	-48.736
4				1.223	-0.165	-1.584	-55.595
5					-10.119	99.305	-66.888
S.E.	gi =2.027		sii =5.233				

Table 59: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for 500 kernels weight in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	2.201	0.070	8.234	11.677	6.118	0.569	-127.234
2		-1.905	2.170	-5.044	15.607	-0.645	-116.520
3			4.280	24.798	8.049	14.047	42.972
4				-2.443	-1.225	1.692	51.641
5					-2.134	0.278	-92.001
S.E.	gi =ns		sii =6.164				

4-2-2-13. Plant yield

General and specific combining abilities have significant effects for plant yield (Appendix 2). Although both gene actions were presented in the inheritance of this trait through the successive generations, the σ^2gca/σ^2sca ratio (0.147 and 0.016) assured the pervasiveness of genes with non-additive effect in the inheritance of plant yield in the original and self-pollinated inbreds, respectively (table 62). The dominance degree results represented an additional indication of the previous conclusion as its value inflated to reach 7.91 in the self-pollinated inbreds after it was 2.61 in their original counterparts. The heritability in its broad sense has not greatly changed after two turns of selfing (94.50% and 95.11%) in disagreement with the narrow sense which its retreatment (21.59% to 2.92%) was really noticeable. Wani et al. (2017) has obtained similar findings, stressing that the non-additive was more present than other types of gene action.

The original inbreds (table 60) exposed a value of 16.402 for inbred 2 as the maximum GCA effect in opposite to a value of -10.317 which was the minimum negative effect observed for inbred 5. After two rounds of selfing, inbred 2 (table 61) took the lead by capturing the highest GCA effect (5.482), whereas, inbred 5 kept grasping the same position with its

lowest negative value (-8.392). The SCA effect was significant in eight out of the ten original hybrids (table 60) scored a range of 32.980 in hybrid 1x5 to -11.311 in hybrid 1x2. The SCA effects were absolutely positive at the next generations to range 43.133 and 4.271 in hybrids 1x4 and 4x5, respectively (table 61).

The most variant original inbred in its GCA effect was inbred 2 with $\sigma^2 gii$ value reached 265.703 (table 60). After two generations, inbred 5 became in the lead with about a quarter of the original value (63.897). The negative variance of the SCA effect was restricted to inbred 2 as reached -73.913 (table 61), while inbred 1 showed the highest positive value (606.971). The positive and negative variance of effect transmitted consistently to the next two generations but with different values to emerge the maximum value of 787.655 in inbred 4 and the minimum value (-15.273) in inbred 2 keeping its negative effect variance.

Table 60: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for plant yield in the original maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	2.132	-11.311	19.385	26.736	32.980	1.219	606.971
2		16.402	3.255	-8.211	7.588	265.703	-73.913
3			-3.855	26.301	1.805	11.537	198.697
4				-4.362	6.150	15.699	342.172
5					-10.317	103.114	233.684
S.E.	gi =2.106		sii =5.439				

Table 61: Estimates of the general gii (diagonal values) and specific sij (above diagonal values) combining abilities for plant yield in the self-pollinated maize population.

Parents	1	2	3	4	5	$\sigma^2 gii$	$\sigma^2 Sii$
1	-3.643	23.585	15.468	43.133	15.704	6.749	650.459
2		5.482	12.692	9.305	10.076	23.532	-15.273
3			2.662	36.728	25.438	0.566	481.697
4				3.890	4.271	8.609	787.655
5					-8.392	63.897	20.734
S.E.	gi =2.949		sii =7.615				

Table 62: Genetic parameters of studied traits in the original and self-pollinated populations of maize.

Studied traits	Population	Genetic Parameters					
		σ^2gca/σ^2sca	σ^2A	σ^2D	$\bar{A}$	$h^2_{.bs}\%$	$h^2_{.ns}\%$
Anthesis days	Original pop.	0.421	1.729	2.053	1.54	94.16	43.05
	Self-poll. Pop.	0.014	0.260	9.227	8.43	94.42	2.58
Silking days	Original pop.	0.966	4.087	2.114	1.02	91.39	60.23
	Self-poll. Pop.	0.071	1.221	8.595	3.75	93.60	11.64
Plant height	Original pop.	0.023	5.415	117.133	6.58	82.94	3.67
	Self-poll. Pop.	0.026	20.385	394.334	6.22	87.14	4.28
Ear height	Original pop.	0.054	7.107	66.115	4.31	86.75	8.42
	Self-poll. Pop.	0.021	9.661	225.286	6.83	89.48	3.68
Leaf area	Original pop.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	Self-poll. Pop.	0.006	53.380	4157.150	12.48	90.40	1.15
Leaf number	Original pop.	0.115	0.146	0.633	2.94	92.66	17.37
	Self-poll. Pop.	0.019	0.062	1.656	7.30	97.19	3.52
Tassel length	Original pop.	0.272	2.840	5.224	1.92	93.90	33.07
	Self-poll. Pop.	0.105	1.596	7.579	3.08	92.66	16.11
Tassel branches number	Original pop.	0.494	2.544	2.572	1.42	79.50	39.52
	Self-poll. Pop.	0.021	0.642	14.946	6.82	93.55	3.85
Ears number	Original pop.	0.167	0.001	0.004	2.44	70.39	17.65
	Self-poll. Pop.	0.152	0.001	0.003	2.57	63.30	14.73
Kernels rows number	Original pop.	1.161	2.127	0.916	0.93	84.59	59.13
	Self-poll. Pop.	5.164	2.324	0.225	0.44	82.17	74.92
Kernels number	Original pop.	0.042	0.446	5.297	4.88	65.90	5.11
	Self-poll. Pop.	0.016	0.555	17.368	7.91	78.89	2.44
500 kernels weight	Original pop.	0.490	109.414	111.606	1.43	86.01	42.58
	Self-poll. Pop.	0.011	3.858	180.866	9.68	78.74	1.64
Plant yield	Original pop.	0.147	171.380	583.609	2.61	95.11	21.59
	Self-poll. Pop.	0.016	40.460	1266.716	7.91	94.50	2.92

Chapter Five

5. Conclusions and Suggestions

1. Self-pollination even for a few generations can result in a considerable alteration in the genetic and epigenetic recital of plant population.
2. The traditional direct relationship between selfing and inbreeding depression has been strengthened by increasing the methylation level in line with the progress of self-pollinated generations.
3. Hybridization is still playing a fundamental role in reducing the level of DNA methylation and contributes efficiently in releasing the gene expression and thus, the outstanding performance of hybrids compared with their parents.
4. The non-additive gene action was prevalent in controlling most of the studied traits in both the original and self-pollinated populations, and selfing increases the predominance of this type of gene action.
5. In general, hybrid vigor and combining ability tend to be more positive with higher values along with selfing generations.

In the light of the above, we may suggest the following:

1. Breeders should pay attention to the propagation method of the inbred lines, because continues practicing of selfing may result in a significant depression in the performance of these lines.
2. There is a need to adopt the epigenetic assessment to obtain more realistic evaluation for new genotypes.
3. Epigenetic variations and their relationship with the management practices should be studied for a deeper understanding of the environmental-epigenetic interference.

Chapter Six

6. References

- Abakemal, D., S. Hussein, J. Derera and K. Semagn. 2014. Genetic purity and patterns of relationships among tropical highland adapted quality protein and normal maize inbred lines using microsatellite markers. Euphytica, 204(1): 49–61.
- Abdul-Hamed, Z.A, I.A. Sarhan and S.A. Abbas. 2017. Combining ability, heterosis and gene action using (line×tester) analysis in corn. The Iraqi J. of Agricultural Sciences, 48(1): 294-301.
- Adebayo, M.A., A. Menkir, E. Blay, V. Gracen and E. Danquah. 2017. Combining ability and heterosis of elite drought-tolerant maize inbred lines evaluated in diverse environments of lowland tropics. Euphytica, 213:43. doi: 10.1007/s10681-017-1840-5.
- Ai-Zhi, L., H. Zhang, Z. Zhang, Y. Tao, B. Yue and Y. Zheng. 2012. Conversion of the statistical combining ability into a genetic concept. J. of Integrative Agriculture, 11(1): 43–52.
- Alfalahi, A.O., M.M. Elsahookie and B.S. Alobaidi. 2012. Molecular variations of maize CMS populations and subpopulations. Iraqi J. Biotech., 11(2): 292-312.
- Al-Falahy, M.A.H. 2015. Estimation combining ability, heterosis and some genetic parameters across four environments using full diallel cross method. Int. J. Pure Appl. Sci. Technol., 26(1): 34-44.
- Ali, S., S.J. Khan, A. Jamil, A.I. Shah and M. Waqas. 2017. Unveiling superior cross combination in maize (*Zea mays* L.). Pure Appl. Biol., 6(2): 676-684. doi: org/10.19045/bspab.2017.60071

- Almelhami, A.S. 2017. Molecular relationship between DNA methylation pattern and hybrid vigor in maize (*Zea mays* L.) using MSAP. Thesis-Dept. of Field Crops Science-College of Agriculture-University of Anbar. p, 92.
- Al-Naggar, A.M.M. and M.M.M. Atta. 2017. Combining ability and heritability of maize (*Zea mays* L.) morphologic traits under water stress and non-stress at flowering stage. Archives of Current Research International, 7(1): 1-16, 2017. doi:10.9734/ACRI/2017/32618
- Alvarez-Venegas, R., C.D. Pen˜a and J.A. Casas-Mollano. 2014. Epigenetics in Plants of Agronomic Importance: Fundamentals and Applications. Springer International Publishing Switzerland. p, 152. doi: 10.1007/978-3-319-07971-4
- Anilkumar, C., H.C. Lohithaswa, S. Ramesh and A.M. Rao. 2017. Exploring relationship between combining ability and stability in maize. Int. J. Curr. Microbiol. App. Sci., 6(7): 2432-2439. doi: org/10.20546/ijcmas.2017.607.345
- Arya, G., A. Maitra and S.A. Grigoryev. 2010. A structural perspective on the where, how, why, and what of nucleosome positioning. J. Biomol. Struct. Dyn., 27:803–820. Doi:
- Aslam, M., Q. Sohail, M.A. Maqbool, S. Ahmad and R. Shahzad. 2017. Combining ability analysis for yield traits in diallel crosses of maize. J. of Animal & Plant Sciences, 27(1): 136-143.
- Bawa, A., I.K. Addai, M.S. Abdulai and A. Issahaku. 2017. Diallel analysis and evaluation of parents and F1 progenies of maize (*Zea mays* L.) for tolerance to drought and *striga hermonthica* (Del.) benth in the Guinea Savanna agro-ecological zone of Ghana. American J. of Agricultural and Biological Sciences, 12 (1): 44-54. doi: 10.3844/ajabssp.2017.44.54.
- Becker, C. and D. Weigel. 2012. Epigenetic variation: Origin and transgenerational inheritance. Curr. Opin. Plant Biol., 15: 562–567. doi: 10.1016/j.pbi.2012.08.004.

- Berger, S.L. 2007. The complex language of chromatin regulation during transcription. Nature, 447: 407–412. doi: 10.1038/nature 05915
- Berretta, J. and A. Morillon. 2009. Pervasive transcription constitutes a new level of eukaryotic genome regulation. EMBO Rep., 10: 973–982.
- Bhandari, H.R., A.N. Bhanu, K. Srivastava, M.N. Singh and A. Hemantaranjan. 2017. Assessment of genetic diversity in crop plants - an overview. Adv. Plants Agric. Res., 7(3):255. doi: 10.15406/apar.2017.07.00255
- Bhatia, G., N. Goyal, S. Sharma, S.k. Upadhyay and K. Singh. 2017. Present scenario of long non-coding RNAs in plants. Non-coding RNA, 3:16. doi:10.3390/ncrna3020016
- Birhanie, Z.M., H.Z. Utta and L.W. Beyene. 2017. Standard heterosis of pipeline maize (*Zea mays* L.) hybrids for grain yield and yield related traits at pawe, northwestern Ethiopia. Int. J. of Plant Breeding and Genetics, 4(1): 249-255.
- Bisen, P., A. Dadheech, O. Nagar and R. K. Meena. 2017. Exploitation of heterosis in single cross hybrids of quality protein maize (*Zea maize* L.) for yield and quality traits. Int. J. of Bio-resource and stress management,8(1):012-019.doi:ORG/10.23910/IJBSM/2017.8.1.1748
- Blevins, T., F. Pontvianne, R. Cocklin, R. Cocklin, R. Podicheti, C. Chandrasekhara, S. Yerneni, C. Braun, B. Lee, D. Rusch, K. Mockaitis, H. Tang and C.S. Pikaard. 2014. A two-step process for epigenetic inheritance in Arabidopsis. Mol. Cell, 54:30–42. doi: 10.1016/j.molcel.2014.02.019.
- Bordersen, P., L. Sakvarelidze-Achard, M. Baruun-Rasmussen, P. Dunoyer, Y.Y. Yamamoto, L. Sieburth, O. Voinnet. 2008. Widespread translational inhibitory plant miRNAs and siRNAs. Science, 320:1185–1190. doi: 10.1126/science.

- Boycheva, I., V. Vassileva and A. Iantcheva. 2014. Histone acetyltransferases in plant development and plasticity. Curr. Genomics, 15(1): 28–37. doi: 10.2174/1389202915011403061127 42
- Bradshaw, J.E. 2017. Plant breeding: Past, present and future. Euphytica, 213: 60. doi:10.1007/s10681-016-1815-y.
- Carthew, R.W. and E.J. Sontheimer. 2009. Origins and mechanisms of miRNAs and siRNAs. Cell, 136:642–655. doi: 10.1016/j.cell.2009.01.035
- Catania, S., P.A. Dumesic, C. Stoddard, S. Cooke, J. Burke, C.A. Cuomo, G.J. Narlikar and H.D. Madhani. 2017. Epigenetic maintenance of DNA methylation after evolutionary loss of the de novo methyltransferase. CSHL, (Article has not peer-reviewed). doi: https://doi.org/10.1101/149385
- Chodavarapu, R.K., S. Feng, B. Ding, S.A. Simon, D. Lopez, Y. Jia, G.L. Wang, B.C. Meyers, S.E. Jacobsen and M. Pellegrini. 2012. Transcriptome and methylome interactions in rice hybrids. Proc. Natl. Acad. Sci., 109(30):12040-5. doi: 10.1073/pnas.1209297109.
- Chow, H.T. and D. W-K. Ng. 2017. Regulation of miR163 and its targets in defense against Pseudomonas syringae in Arabidopsis thaliana. Scientific Reports, 7: 46433. doi :10.1038/srep46433
- Clapier, C.R., J. Iwasa, B.R. Cairns and C.L. Peterson. 2017. Mechanisms of action and regulation of ATP-dependent chromatin-remodelling complexes. Nature Reviews Molecular Cell Biology, 18: 407–422. doi: 10.1038/nrm.2017.26
- Dapp, M., J. Reinders, A. Bédiée, C. Balsera, E. Bucher, G. Theiler, C. Granier and J. Paszkowski. 2015. Nature Plants, 1. doi:10.1038/nplants.2015.92
- Dar, Z.A., A.A. Lone, N.S. Khuroo, G. Ali, I. Abidi, M.A. Ahangar, M.A. Wani, A.B. Yasin, A. Gazal, R.A. Lone, N. Yousuf and S. Gulzar. 2017. Line x Tester analysis in maize (*Zea mays* L.) for various morpho-agronomic traits

under temperate conditions. Int. J. Curr. Microbiol. App. Sci., 6(7):1430-1437. doi: org/10.20546/ijcmas.2017.607.171

- Darwin, C.R. 1876. The effects of cross- and self-fertilisation in the vegetable kingdom. New Phytol., 198: 71–78. doi:10.2135/cropsci1977.0011183X001700010014x.
- Efendi, R., S. Sunarti, Y. Musa, M.F. Bdr, M.D. Rahim and M. Azrai1. 2015. Selection of homozygosity and genetic diversity of maize inbred using simple sequence repeats (SSRs) marker. Int. J. Curr. Res. Biosci. Plant Biol., 2(3): 19-28.
- Eichten, S. R., R. A. Swanson-Wagner, J.C. Schnable, A.J. Waters, P.J. Hermanson, S. Liu, C-T. Yeh, Y. Jia, K. Gendler, M. Freeling, P.S. Schnable, M.W. Vaughn and N.M. Springer. 2011. Heritable epigenetic variation among maize inbreds. PLoS Genet., 7:e1002372. doi.org/10.1371/journal.pgen.1002372
- Eichten, S.R., R. Briskine, J. Song, Q. Li, R. Swanson-Wagner, P.J. Hermanson, A.J. Waters, E. Starr, P.T. West, P. Tiffin, C.L. Myers, M.W. Vaughn and N.M. Springer. 2013. Epigenetic and genetic influences on DNA methylation variation in maize populations. The Plant Cell, 25: 2783–2797.
- Excoffier, L. 2001. Analysis of Population Subdivision. Handbook of Statistical Genetics. p, 271-307.
- Feng, S.Q., X.L. Chen, S.J. Wu and X.S. Chen 2015. Recent advances in understanding plant heterosis. Agricultural Sciences, 6:1033-1038. doi.org/10.4236/as.2015.69098
- Fortes, A.M. and P. Gallusci. 2017. Plant stress responses and phenotypic plasticity in the epigenomics era: Perspectives on the grapevine scenario, a model for perennial crop plants. Front. Plant Sci., 8:82. doi: 10.3389/fpls.2017.00082

- Ganapathy, M. 2016. Plants as Bioreactors. Adv. Tech. Biol. Med., 4(1):1-9. 4:161. doi: 10.4172/2379-1764.1000161.
- Geoffrey, G.W., H. Wang, D.F. Heiter and K.D. Lunnen. 2012. Restriction enzymes in microbiology, biotechnology and biochemistry. Encuentro, 93: 19-48.
- Goldberg, E.E., J.R. Kohn, R. Lande, K.A. Robertson, S.A. Smith and B. Igic. 2010. Species selection maintains self-incompatibility. Science, 330: 493–495.
- Goldstein, M., F.A. Derheimera, J. Tait-Muldera and M.B. Kastana. 2013. Nucleolin mediates nucleosome disruption critical for DNA double-strand break repair. PNAS, 110(42): 16874–16879. doi: 10.1073/pnas.1306160110
- Goulet, B.G., F. Roda and R. Hopkins. 2017. Hybridization in plants: Old ideas, new techniques. Plant Physiology, 173: 65–78. doi: 10.1104/pp.16.01340.
- Grabsztunowicz, M., M.M. Koskela and P. Mulo. 2017. Post-translational modifications in regulation of chloroplast function: Recent advances. Front Plant Sci., 8: 240. doi: 10.3389/fpls.2017.00240
- Griffing, B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Australian J. of Bio. Sci., 9(4) 463 - 493. doi.org/10.1071/BI9560463.
- Groszmann, M., I.K. Greaves, R. Fujimoto, W.J. Peacock and E.S. Dennis. 2013. The role of epigenetics in hybrid vigour. Trends Genet., 29(12): 684–690. doi: 10.1016/j.tig.2013.07.004.
- Guleria, P., M. Mahajan, J. Bhardwaj, S.K. Yadav. 2011. Plant small RNAs: Biogenesis, mode of action and their roles in abiotic stresses. Genomics, Proteomics & Bioinformatics, 9(6):183–199. doi: org/10.1016/S1672-0229(11)60022-3
- Gutzat, R. and M. Scheid. 2012. Epigenetic responses to stress: Triple defense? Curr. Opin. Plant Biol., 15: 568–573. doi: 10.1016/j.pbi.2012.08.007.

- Hartfield, M., T. Bataillon and S. Glémin. 2017. The evolutionary interplay between adaptation and self-fertilization. Trends Genet., 33(6):420–431. doi: 10.1016/j.tig.2017.04.002
- He, G., X. Zhu, A.A. Elling, L. Chen, X. Wang, L. Guo, M. Liang, H. He, H. Zhang, F. Chen, Y. Qi, R. Chen, .X.W. Deng. 2010. Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. The Plant Cell, 22:17-33. doi.org/10.1105/tpc.109.072041
- Herbst, R.H., D. Bar-Zvi1, S. Reikhav, I. Soifer, M. Breker, G. Jona, E. Shimoni, M. Schuldiner, A.A. Levy and N. Barkai. 2017. Heterosis as a consequence of regulatory incompatibility. BMC Biology, 15:38. doi: 10.1186/s12915-017-0373-7
- Holoch, D. and D. Moazed. 2015. RNA-mediated epigenetic regulation of gene expression. Nat. Rev. Genet., 16(2):71-84. doi: 10.1038/nrg3863
- Hubbs, T. 2017. Weekly Outlook: 2017 Corn Prospects. Department of Agricultural and Consumer Economics, University of Illinois at Urbana-Champaign. p,31. http://farmdocdaily.illinois.edu/2017/02/2017-corn-prospects.html.
- Hussein, M.A., S.E. Haji and S. Ramadan. 2015. Estimation of combining ability in maize lines using a diallel cross. J. Pure Appl. Sci. Technol., 27(2): 87-95.
- Jin, J., Y. Sun, J. Qu, R. syah, C-H. Lim, Y. Alfiko, N. Rahman, A. Suwanto, G. Yue, L. Wong, N-H. Chua and J. Ye. 2017. Transcriptome and functional analysis reveals hybrid vigor for oil biosynthesis in oil palm. Scientific Reports, 7(439):1-12. doi:10.1038/s41598-017-00438-8.
- Jin, L., G. Li, D. Yu, W. Huang, C. Cheng, S. Liao, Q. Wu and Y. Zhang. 2017. Transcriptome analysis reveals the complexity of alternative splicing regulation in the fungus *Verticillium dahliae*. BMC Genomics, 18:130. doi.org/10.1186/s12864-017-3507-y

- Kamara, M.M. 2016. Combining ability and genetic diversity using SSR markers for some maze inbred lines. Egypt. J. Plant Breed., 20(2):373 – 395.
- Kardos, M., H.R. Taylor, H. Ellegren, G. Luikart and F.W. Allendorf. 2016. Genomics advances the study of inbreeding depression in the wild. Evolutionary Applications, 9: 1205–1218. doi: 10.1111/eva.12414
- Katba, P.J., R.G. Hadiya, V.N. Kapadia, D.C. Patel and A.D. Patel. 2017. Genetics studies on yield and pharmaceutical quality parameters in tobacco (*Nicotiana rustica* L.). J. of Pharmacognosy and Phytochemistry, 6(1): 399-404.
- Kawamura, K., T. Kawanabe, M. Shimizu, K. Okazaki, M. Kaji, E.S. Dennis, K. Osabe and R. Fujimoto. 2016. Genetic characterization of inbred lines of Chinese cabbage by DNA markers; towards the application of DNA markers to breeding of F1 hybrid cultivars. Data in Brief, 6:229–237. doi:org/10.1016/j.plgene.2015.10.003
- Khotyleva, L.V., A.V. Kilchevsky and M.N. Shapturenko. 2017. Theoretical aspects of heterosis. Russian Journal of Genetics: Applied Research, 7(4): 428-439. doi:10.1134/S2079059717040049.
- Kitagawa, M. and D. Jackson. 2017. Plasmodesmata-mediated cell-to-cell communication in the shoot apical meristem: How stem cells talk. Plants, 6:12. doi:10.3390/plants6010012
- Köhler, C. and N. Springer. 2017. Plant epigenomics-deciphering the mechanisms of epigenetic inheritance and plasticity in plants. Genome Biol., 18: 132. doi: 10.1186/s13059-017-1260-9
- Konate, L., B. Baffour and D. Traore. 2017. Combining ability and heterotic grouping of early maturing pro vitamin A maize inbreds across Striga infested and optimal growing environments. J. of Agriculture and Environment for International Development, 111(1): 157-173. doi: 10.12895/jaeid.20171.572.

- Lämke, J. and I. Bäurle. 2017. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biology, 18:124. doi: 10.1186/s13059-017-1263-6
- Laosuwan, P. and R.E. Atkins. 1977. Estimates of combining ability and heterosis in converted exotic sorghums. Crop Science J., 17(1): 47-50.
- Larièpe, A., L. Moreau, J. Laborde, C. Bauland, S. Mezmouk, L. Décousset, T. Mary-Huard, J. B. Fiévet, A. Gallais, P. Dubreuil and A. Charcosset. 2017. General and specific combining abilities in a maize (*Zea mays* L.) test-cross hybrid panel: relative importance of population structure and genetic divergence between parents. Theoretical and Applied Genetics, 130(2): 403–417.
- Lauria, M., S, Piccinini, R. Pirona, G. Lund, A. Viotti and M. Motto. 2014. Epigenetic variation, inheritance, and parent-of-origin effects of cytosine methylation in maize (*Zea mays*). Genetics, 196: 653–666.
- Lauss, K. 2017. Phenotypic variation in plants: Roles for epigenetics. PhD Dissertation, Faculty of Science (FNWI), University of Amsterdam, Holland. p,310.
- Law, J.A. and S.E. Jacobsen. 2010. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat. Rev. Genet., 11(3): 204220. doi: 10.1038/nrg2719
- Lee, E.A., M.J. Ash and B. Good. 2007. Re-examining the relationship between degree of relatedness, genetic effects and heterosis in Maize. Crop Sci. 47: 629-635.
- Lee, B.B., J.H. Kim and T.S. Kim. 2017. Fine-tuning of gene expression dynamics by the Set2-Rpd3S pathway. BMB Rep., 50(4): 162-163. doi: 10.1038/ncomms13534
- Li, Y., X-M. Dong, F. Jin, Z. Shen, Q. Chao and B-C. Wang. 2017. Histone acetylation modifications affect tissue-dependent expression of poplar

homologs of C4 photosynthetic enzyme genes. Front Plant Sci., 8:950. doi: 10.3389/fpls.2017.00950

- Li, Z., L. Coffey, J. Garfin, N.D. Miller, M.R. White, E.P. Spalding, N. de Leon, S.M. Kaeppler, P.S. Schnable, N.M. Springer and C.N. Hirsch. 2017. Genotype-by-environment interactions affecting heterosis in maize. doi: http://dx.doi.org/10.1101/131342.
- Liton, M.M.U.A., M.S.R. Bhuiyan, N. Zeba and M.H. Rashid. 2017. Estimation of heterosis for yield and its attributes in Brassica rapa L. Asian Research J. of Agriculture, 4(4): 1-13.
- Liu, D., R. Mewalal, R. Hu, G.A. Tuskan and X. Yang. 2017. New technologies accelerate the exploration of non-coding RNAs in horticultural plants. Horticulture Research, 4: 17031. doi:10.1038/hortres.2017.31
- Liu, J., H. Wang and N.H. Chua. 2015. Long noncoding RNA transcriptome of plants. Plant Biotech. J., 13: 319–328.
- Liu, T.J., L.F. Sun, X.H. Shan, Y. Wu, S.Z. Su, S.P. Li, H.K. Liu, J.Y. Han and Y.P. Yuan. 2014. Analysis of DNA methylation patterns and levels in maize hybrids and their parents. Genet. Mol. Res., 13(4): 8458-8468.
- Liu, X., S. Yang, C-W. Yu, C-Y. Chen and K. Wu. 2017. Histone acetylation and plant development (Chapter six). The Enzymes, 40: 173-199. doi: org/10.1016/bs.enz.2016.08.001.
- Lu, X., X. Wang, X. Chen, N. Shu, J. Wang, D. Wang, S. Wang, W. Fan, L. Guo, X. Guo and W. Ye. 2017. Single-base resolution methylomes of upland cotton (*Gossypium hirsutum* L.) reveal epigenome modifications in response to drought stress. BMC Genomics, 18(297): 1-14. doi: 10.1186/s12864-017-3681-y
- Lukens, L.N. and S. Zhan. 2007. The plant genome's methylation status and response to stress: implications for plant improvement. Curr. Opin. Plant Biol. 10: 317-322.

- Ma, X., S. Lv, C. Zhang and C. Yang. 2013. Histone deacetylases and their functions in plants. Plant Cell Rep., 32: 465–478. doi: 10.1007/s00299-013-1393-6
- Marcon, C., A. Paschold, W.A. Malik, A. Lithio, J.A. Baldauf, L. Altrogge, N. Opitz, C. Lanz, H. Schoof, D. Nettleton, H-P. Piepho and Frank Hochholdinger. 2017. Stability of single-parent gene expression complementation in maize hybrids upon water deficit stress. Plant Physiol., 173(2): 1247–1257. doi: 10.1104/pp.16.01045
- Matin, M.Q.I., M.d.G. Rasul, A.K.M. Aminul Islam, M. A. K. Mian, N.A. Ivy and J. Ahmed. 2017. Combining ability and heterosis in maize (*Zea mays* L.). American J. of Bio Science, 4(6): 84-90. doi: 10.11648/j.ajbio.20160406.12.
- Matsui, A., A.H. Nguyen, K. Nakaminami and M. Seki. 2013. Arabidopsis non-coding RNA regulation in abiotic stress responses. Int. J. Mol. Sci., 14: 22642–22654.
- McKeown, P.C. and C. Spillane. 2014. Landscaping plant epigenetics. Methods Mol. Biol., 1112: 1–24. doi: 10.1007/978-1-62703-773-0_1.
- Meena, A.K., D. Chouhan, D. Singh and V. Nepalia. 2017. Response of pop corn (*Zea mays* L. everta) varieties to varying plant densities and fertility levels. Indian Journal of Agronomy, 62(1):43-46.
- Meng, F.R., Y.C. Li, J. Yin, H. Liu, X.J. Chen, Z.F. Ni and Q.X. Sun. 2012. Analysis of DNA methylation during the germination of wheat seeds. Bio. Plant arum 56:269–275
- Muhammad, R.W., A. Qayyum, M.Q. Ahmad, A. Hamza, M. Yousaf,B. Ahmad, M. Younas, W. Malik, S. Liaqat and E. Noor. 2017. Characterization of maize genotypes for genetic diversity on the basis of inter simple sequence repeats. Genetics and Molecular Research, 16(1): 16019438. doi: doi.org/10.4238/gmr16019438

- Mulualem, T. and M. Abate. 2016. Heterotic response in major cereals and vegetable crops. Int. J. Plant Breed. Genet., 10: 69-78. doi: 10.3923/ijpbg.2016.69.78.
- Pabendon, M.B., Azrai, M. Mejaya and M.J. Sutrisno. 2008. Genetic diversity of QPM and normal maize inbreds as revealed by SSR markers and its relationship with the hybrid performance. J. Agro. Biogen., 4(2): 77-82.
- Pabendon, M.B., M.J. Mejaya, J. Koswara and H. Aswidinnoor. 2010. Correlation between genetic distances based on microsatellite marker in maize inbred with seed weight of F1. Penelitian Pertanian Tanaman Pangan, 29(1): 11-17.
- Paige, K.N. 2010. The Functional genomics of inbreeding depression: A new approach to an old problem. BioScience, 60(4): 267–277.
- Pemberton, J.M., P.E. Ellis, J.G. Pilkington and C. Béréno. 2017. Inbreeding depression by environment interactions in a free-living mammal population. Heredity, 118: 64–77. doi:10.1038/hdy.2016.100
- Peng, M., P. Ying, X. Liu, C. Li, R. Xia, J. Li and M. Zhao. 2017. Genome-Wide identification of histone modifiers and their expression patterns during fruit abscission in Litchi. Front. Plant Sci., 8(639): 1-16. doi: 10.3389/fpls.2017.00639
- Postnote. 2017. New Plant Breeding Techniques. The Parliamentary Office of Science and Technology, Westminster, London-UK. 548: 1-4.
- Prathiba, K.Y., S. Usha, B. Suchithra, M.N. Jyothi, V.R. Devaraj and R. Nageshbabu. 2017. Computational identification of miRNAs and their targets from Niger (*Guizotia abyssinica*). J. Appl. Bio. & Biotech., 5(2): 53-58. doi: 10.7324/JABB.2017.50208
- Qi, X., Z.H. Li, L.L. Jiang, X.M. Yu, F. Ngezahayo and B. Liu. 2010. Grain-yield heterosis in *Zea mays* L. shows positive correlation with parental difference in CHG methylation. Crop Sci. 50(10): 2338-2346.

- Rajewsky, N., S. Jurga, J. Barciszewski. 2017. Plant Epigenetics. Springer International Publishing. Gewerbestrasse 11, 6330 Cham, Switzerland. p, 536. doi: 10.1007/978-3-319-55520-1
- Raut, D.M., A.B. Tamnar, S.V. Burungale and P.L. Badhe. 2017. Half diallel analysis in cowpea *Vigna unguiculata* (L.) Walp.]. Int. J. Curr. Microbiol. App. Sci., 6(7):1807-1819. doi:org/10.20546/ijcmas.2017.607.218.
- Reyna-Lopez, G.E., J. Simpson and J. Ruiz-Herrera. 1997. Differences in DNA methylation patters are detectable during the dimorphic transition of fungi by amplification of restriction polymorphisms. Mol. Gen. Genet. 253: 703–710.
- Rodrigues, J.A. and D. Zilberman. 2015. Evolution and function of genomic imprinting in plants.Genes & Dev., 29: 2517-2531. doi: 10.1101/gad.269902.115.
- Ryder, P., P.C. McKeown, A. Fort and C. Spillane. 2014. Epigenetics and Heterosis in Crop Plants. In: Epigenetics in Plants of Agronomic Importance: Fundamentals and Applications. Springer International Publishing Switzerland. p, 152. doi: 10.1007/978-3-319-07971-4
- Santos, A.P., L.J. Ferreira and M.M. Oliveira. 2017. Concerted flexibility of chromatin structure, methylome, and histone modifications along with plant stress responses. Biology (Basel), 6(1): 3. doi: 10.3390/biology6010003
- Sarkies, P. and E.A. Miska. 2014. Small RNAs break out: the molecular cell biology of mobile small RNAs. Nat Rev Mol Cell Biol., 15(8):525-35. doi: 10.1038/nrm3840.
- Schmitz, R.J. and J.R. Ecker. 2012. Epigenetic and epigenomic variation in *Arabidopsis thaliana*. Trends Plant Sci., 17: 149–154. doi: 10.1016/j.tplants.2012.01.001.

- Schulz, B., R.L. Eckstein and W. Durka. 2013. Scoring and analysis of methylation- sensitive amplification polymorphisms for epigenetic population studies. *Molecular Ecology Resources*, 13: 642–653.
- Secco, D., J. Whelan, H. Rouached and R. Lister. 2017. Nutrient stress-induced chromatin changes in plants. Current Opinion in Plant Biology, 39: 1–7. doi: org/10.1016/j.pbi.2017.04.001.
- Shan, X., X. Wang, G. Yang, Y. Wu, S. Su, S. Li, H. Liu and Y. Yuan. 2013. Analysis of the DNA methylation of maize (*Zea mays* L.) in response to cold stress based on methylation-sensitive amplifid polymorphisms. J. Plant Biol 56:32–38.
- Shen, H., H .He, J. Li, W. Chen, X. Wang, L. Guo, Z. Peng, G. He, S. Zhong, Y. Qi, W. Terzaghi and X.W. Deng. 2012. Genome-wide analysis of DNA methylation and gene expression changes in two Arabidopsis ecotypes and their reciprocal hybrids. The Plant Cell, 24, 875-892.http://dx.doi.org/10.1105/tpc.111.094870
- Shen, Y., S. Sun, S. Hua, E. Shen, C.Y. Ye, D. Cai, M.P. Timko, Q.H. Zhu and L. Fan. 2017. Analysis of transcriptional and epigenetic changes in hybrid vigor of allopolyploid *Brassica napus* uncovers key roles for small RNAs. The Plant Journal, doi: 10.1111/tpj.13605.
- Shuro, A.R. 2017. Review paper on approaches in developing inbred lines in cross-pollinated crops. Biochemistry and Molecular Biology, 2(4): 40-45. doi: 10.11648/j.bmb.20170204.12
- Singh, R.K. and B.D. Chaudhary. 1985. Biometrical methods in quantitative genetic analysis. Kalyani Publ., Ludhiana, New Delhi, India. pp, 300.
- Song, G.S., H.L. Zhai, Y.G. Peng, L. Zhang, G. Wei, X.Y. Chen, Y.G. Xiao, L. Wang, Y.J. Chen, B. Wu, B. Chen, Y. Zhang, H. Chen, X.J. Feng, W.K. Gong, Y. Liu, Z.J. Yin, F. Wang, G.Z. Liu, H.L. Xu, X.L. Wei, X.L. Zhao, P.B. Ouwerkerk, T. Hankemeier, T. Reijmers, R. van der Heijden, C.M. Lu,

M. Wang, J. van der Greef and Z. Zhu. 2010. Comparative transcriptional profiling and preliminary study on heterosis mechanism of super-hybrid rice. Mol Plant 3:1012–1025. doi: 10.1093/mp/ssq046.

- Sorkheh, K., M.K. Dehkordi, S. Ercisli, A. Hegedus and J. Halász. 2017. Comparison of traditional and new generation DNA markers declares high genetic diversity and differentiated population structure of wild almond species. Scientific Reports, 7:5966. doi: 10.1038/s41598-017-06084-4
- Sprague, G.F. and L.A. Tatum. 1942. General vs. specific combining ability in single crosses of corn. J. Amer. Soc. Agron., 34: 923–32.
- Springer, N.M., and R.J. Schmitz. 2017. Exploiting induced and natural epigenetic variation for crop improvement. Nature Reviews Genetics, doi:10.1038/nrg.2017.45.
- Stewart, D.W. and L.M. Dwyer. 1999. Mathematical characterization of leaf shape and area of maize hybrids. Crop Science J., 39(2): 422-427. doi:10.2135/cropsci1999.0011183X0039000200021x
- Sunkar, R., Y-F. Li and G. Jagadeeswaran. 2012. Functions of microRNAs in plant stress responses. Trends Plant Sci., 17: 196–203.
- Technical Whitepaper (6). 2005. Euclidean Distance Raw, Normalized and Double Scaled Coefficient. pp, 26.
- Vaucheret, H., A.C. Mallory and D.P. Bartel. 2006. AGO1 homeostasis entails co expression of MIR168 and AGO1 and preferential stabilization of miR168 by AGO1. Mol. Cell., 22:129–136. doi: 10.1016/j.molcel.2006.03.011
- Vergara, Z. and C. Gutierrez. 2017. Emerging roles of chromatin in the maintenance of genome organization and function in plants. Genome Biology, 18:96. doi: 10.1186/s13059-017-1236-9
- Vergeer, P., N. Wagemaker and N.J. Ouborg. 2012. Evidence for an epigenetic role in inbreeding depression. Biol. Lett., 8: 798–801. doi:10.1098/rsbl.2012.0494

- Wang, Z., H. Cao, F. Chen and Y. Liu. 2014. The roles of histone acetylation in seed performance and plant development. Plant Physiol. Biochem., 84:125-33. doi: 10.1016/j.plaphy.2014.09.010.
- Wani, M.A., S.A. Wani, Z.A. Dar, A.A. Lone, I. Abedi and A. Gazal. 2017. Combining ability analysis in early maturing maize inbred lines under temperate conditions. Int. J. Pure App. Biosci., 5(2): 456-466. doi.org/10.18782/2320-7051.2538.
- Wolde, L., T. Keno, B. Tadesse, M. Worku and D. Wogari. 2017. Combing ability analysis of among early generation maize inbred lines. J. Agric. Sci. 27(2): 49-60.
- Wu, G. 2013. Plant microRNAs and development. J. Genet. Genomics, 40:217–230
- Xiao, J., R. Jin and D. Wagner. 2017. Developmental transitions: integrating environmental cues with hormonal signaling in the chromatin landscape in plants. Genome Biology, 18:88. doi: 10.1186/s13059-017-1228-9
- Xu, J., Y. Li, Y. Wang, X. Liu and X-G. Zhu. 2017. Altered expression profiles of microRNA families during de-etiolation of maize and rice leaves. BMC Res. Notes, 10:108. doi: 10.1186/s13104-016-2367-x
- Yang, S., C. Li, L. Zhao, S.Gao, J. Lu, M. Zhao, C-Y. Chen, X. Liu, M. Luo, C. Yang and K. Wu. 2015. The Arabidopsis SWI2/SNF2 chromatin remodeling ATPase BRAHMA targets directly to PINs and is required for root stem cell niche maintenance. Plant Cell, 27:1670–1680. doi: 10.1105/tpc.15.00091.
- Yang, W., X. Yu, W. Yang and B. Liu. 2011. Parental epigenetic difference in DNA methylation-level may play contrasting roles for different agronomic traits related to yield heterosis in maize. African Journal of Biotechnology, 10(46): 9253-9263.

- Yang, X.,W. Yu, L. Shi, L. Sun, J. Liang, X. Yi, Q. Li, Y. Zhang, F. Yang, X. Han, D. Zhang, J. Yang and Z. Yao. 2011. HAT4, a Golgi apparatus-anchored B-type histone acetyltransferase, acetylates free histone H4 and facilitates chromatin assembly. Molecular cell, 44(1):39–50. doi: org/10.1016/j.molcel.2011.07.032
- Yu, Y., X. Yang, H. Wang, F. Shi, Y. Liu, J. Liu, L. Li, D. Wang and B. Liu. 2013. Cytosine methylation alteration in natural populations of *Leymus chinensis* induced by multiple abiotic stresses. PLoS ONE 8:e55772
- Zannas, A.S. and G.P. Chrousos. 2017. Epigenetic programming by stress and glucocorticoids along the human lifespan. Molecular Psychiatry, 22: 640-646. doi:10.1038/mp.2017.35
- Zhang, D., Y. Li, X. Zhang, P. Zha and R. Lin. 2017. The SWI2/SNF2 chromatin-remodeling ATPase BRAHMA regulates chlorophyll biosynthesis in Arabidopsis. Molecular Plant, 10: 155–167. doi: org/10.1016/j.molp.2016.11.003
- Zhang, X., L. Lv, C. Lv, B. Guo and R. Xu. 2015. Combining ability of different agronomic traits and yield components in hybrid barley. PLoS ONE, 10(6): e0126828. doi:10.1371/journal.pone.0126828.
- Zhang, X., S.H. Shiu, A. Cal and J.O. Borevitz. 2008. Global analysis of genetic, epigenetic and transcriptional polymorphisms in Arabidopsis thaliana using whole genome tiling arrays. PLoS Genet 4(3): e1000032. doi: org/10.1371/journal.pgen.1000032
- Zhang, Y-C. and Y-Q. Chen. 2017. Epigenetic Regulation by Noncoding RNAs in Plant Development (Chapter). In Rajewsky, N., S. Jurga, J. Barciszewski. Plant Epigenetics. Springer International Publishing. Gewerbestrasse 11, 6330 Cham, Switzerland. pp, 183-198. doi: 10.1007/978-3-319-55520-1

- Zhao, D., K. Chong and R. Palanivelu. 2017. Editorial: Molecular and cellular plant reproduction. Front. Plant Sci., 8:199. doi: 10.3389/fpls.2017.00199
- Zhao, X.X., Y. Chai and B. Liu. 2007. Epigenetic inheritance and variation of DNA methylation level and pattern in maize intra-specific hybrids. Plant Sci., 172: 930–938.
- Zhao, Y. and D.X. Zhou. 2012. Epigenomic modification and epigenetic regulation in rice. J. Genet. Genomics, 39:307–315. doi: 10.1016/j.jgg.2012.02.009
- Zhou, S., W. Jiang, F. Long, S. Cheng, W. Yang, Y. Zhao and D-X. Zhou. 2017. Rice homeodomain protein WOX11 recruits a histone acetyltransferase complex to establish programs of cell proliferation of crown root meristem. The Plant Cell, 29: 1088–1104. doi: 10.1105/tpc.16.00908.
- Zilberman, D. 2008. The evolving functions of DNA methylation. Curr. Opin. Plant Biol. 11: 554-559.

Appendix 1: Analysis ofMolecular variance (AMOVA) for Methylation Sensitiv Loci (MSL) in the original and self-pollinated populations of maize using MSAP.

Source of Varaince	df	MS	Variance
Original Pop. **vs** Self-pollinated Pop.	1	6.254	0.2172
Genotypes (Original Pop. **and** Self-pollinated Pop.)	28	2.996	2.996

Appendix 2: Mean squares of the studied traits in the original and self-pollinated populations of maize.

Source of variance		Replicates		Genotypes	Exp. error	GCA	SCA	Exp. error
Studied traits	Population	df	2	14	28	4	10	28
Anthesis days	Original pop.	5.49		11.03*	0.70	7.151*	2.288*	0.234
	Self-poll. Pop.	0.47		22.34*	1.68	1.598*	9.787*	0.560
Silking days	Original pop.	7.80		20.30*	1.75	16.932*	2.698*	0.584
	Self-poll. Pop.	0.47		24.62*	2.01	5.556*	9.267*	0.671
Plant height	Original pop.	23.03		345.17*	75.60	46.862^{ns}	142.332*	25.200
	Self-poll. Po.p	518.13		1098.50*	183.61	142.741^{ns}	455.536*	61.202
Ear height	Original pop.	18.86		199.60*	33.56	39.614*	77.302*	11.187
	Self-poll. Pop.	141.99		598.78*	82.90	66.279^{ns}	252.920*	27.634
Leaf area	Original pop.	376444.15		335197.28	349534.75	ns	ns	ns
	Self-poll. Pop.	27631.41		10432.67*	1341.48	660.678^{ns}	4604.310*	447.160
Leaf number	Original pop.	0.44		2.04*	0.18	0.645*	0.694*	0.062
	Self-poll. Pop.	0.78		3.91*	0.15	0.298*	1.706*	0.050
Tassel length	Original pop.	1.98		22.50*	1.57	11.884*	5.747*	0.523
	Self-poll. Pop.	0.96		23.89*	2.18	7.110*	8.306*	0.727
Tassel branches number	Original pop.	11.30		18.19*	3.96	11.494*	3.892*	1.320
	Self-poll. Pop.	7.65		37.45*	3.23	3.643*	16.022*	1.075
Ears number	Original pop.	0.02		0.02*	0.01	0.008*	0.007*	0.002
	Self-poll. Pop.	0.00		0.02*	0.01	0.006^{ns}	0.006*	0.002
Kernels rows number	Original pop.	2.67		10.92*	1.66	9.063*	1.470*	0.554
	Self-poll. Pop.	6.50		10.11*	1.66	9.850*	0.778^{ns}	0.553
Kernels number	Original pop.	4.07		21.80*	8.92	4.754^{ns}	8.269*	2.972
	Self-poll. Pop.	10.25		53.51*	14.39	7.015^{ns}	22.163*	4.795
500 kernels weight	Original pop.	201.17		722.10*	107.81	473.593*	147.543*	35.938
	Self-poll. Pop.	33.90		550.39*	149.59	65.294^{ns}	230.730*	49.864
Plant yield	Original pop.	326.00		1954.64*	116.46	724.341*	622.430*	38.821
	Self-poll. Pop.	189.20		3081.42*	228.30	237.942*	1342.817*	76.100