DNA METHYLATION AND FESOD GENE EXPRESSION AFFECTED BY PLANT DENSITY IN *ZEA MAYS* L.

***M. H. Shenawa** **A. O. Alfalahi**
Researcher **Assist. Prof.**
Dept. of Field Crops – Coll. of Agric. Univ of Anbar
ag.ayoob.obaid@uoanbar.edu.iq

ABSTRACT

Methylation Sensitive Amplification Polymorphism (MSAP) was used to characterize the alterations in DNA methylation in maize (*Zea mays* L.) inbred lines and their half-daillels affected by plant densities (213333 plant h-1 and 13333 plant h-1). The two restriction was enzymes (HpaII and MspI) succeeded in diagnosing a total of 23 specific loci, most of (22 loci) were Methylation Sensitive Loci (MSL), while the only one NML (No Methylated Loci) was monomorphic. Thirteen out of 22 MSL loci polymorphic, recording a were polymorphism percentage of 59%. Results of FeSOD gene expression cleared the different response of maize inbreds and hybrids to high plant density stress. Generally, the expression of the targeted gene was increased in plants submitted to high plant density stress compared with low density. The inbred 3 and its single hybrid 1×3 achieved the highest level of gene expression under high planting density (5505.7 and 21098.6 copy, respectively), meanwhile, inbred 5 and it's single hybrid 4×5 gained the maximum level of FeSOD expression at the low plant density (8317.6 and 6862.1 copy, respectively). The response reached to its maximum limit in many of those genotypes, some other genotypes showed relatively steady performance along with higher stress, such as parent 1, that gave the lowest number of gene copies in both, high and low plant density (1375.8 and 1569.5 copy, respectively).

keywords: Zea mays, plant density, msap, dna methylation, fesod gene expression.
***Part of M.Sc. thesis of the 1st author.**

ميثلة DNA والتعبير الجيني لجين FeSOD تحت تأثير الكثافة النباتية في الذرة الصفراء

***مهند حامد شناوه** **ايوب عبيد الفلاحي**
الباحث **أستاذ مساعد**
قسم المحاصيل الحقلية – كلية الزراعة – جامعة الانبار

المستخلص

اظهرت نتائج التحليل الجزيئي لتقنية MASP (Methylation Polymorphism Amplified Sensitive) باستعمال انزيمي القطع HpaII و MspI لنمط ميثلة DNA وللبادئين المستعملين في تشخيص مواقع القطع في جينومات التراكيب الوراثية تحت تاثير الكثافة النباتية العالية (213333 نبات هـ$^{-1}$) والواطئة (13333 نبات هـ$^{-1}$)، نجح الانزيمين المذكورين في تشخيص ما مجموعه 23 موقعا متخصصا (Specific Loci)، كانت جلها حساسة للتميثل MSL (Methylation–Susceptible Loci) بلغت 22 موقعا، بينما كان موقعا واحدا غير حساسا للتميثل NML (No Methylated Loci). تباين شكل 13 من مواقع القطع الحساسة للتميثل (Polymorphic MSL Loci) مسجلة نسبة مئوية بلغت 59%، في حين تماثلت مواقع القطع غير المتميثلة (Polymorphic NML Loci). اوضحت نتائج تعبير جين Fe–SOD المضاد للاكسدة استجابة نبات الذرة الصفراء على جزيئيا لإجهاد الكثافة النباتية العالية، فقد ازداد تعبير هذا الجين في التراكيب الوراثية المعرضة لاجهاد الكثافة النباتية العالية مقارنة بالكثافة الواطئة، فقد أعطى الأب رقم 3 والهجين رقم 7 في الكثافة النباتية العالية أعلى مستوى للتعبير الجيني (5505.7 و 21098.6 نسخة) بالتتابع، بينما عند الكثافة النباتية الواطئة تفوق الاب 5 والهجين 15 باعطاء اعلى مستوى للتعبير الجيني (8317.6 و6862.1 نسخة) ففي الوقت الذي كانت فيه استجابة عدد من تلك التراكيب على أشدها، أظهر عدد آخر من التراكيب أداءً ثابتاً نسبياً بتأثير إجهاد الكثافة النباتية كما في الأب 1، الذي كان تعبير جين Fe–SOD في خلاياه هو الأوطأ في كلا الكثافتين، العالية والواطئة (1375.8 و1569.5 نسخة) بالتتابع.

الكلمات المفتاحية: الذرة الصفراء، الكثافة النباتية، MSAP، ميثلة الـ DNA، التعبير الجيني لجين FeSOD
***جزء من رسالة ماجستير الباحث الاول.**

***Received:25/5/2018, Accepted:24/9/2018**

INTRODUCTION

Plant density is a vital practice that has received considerable attention from plant breeders within the vertical expansion of crop cultivation to increase grain yield. Adaptation to such practices is a key factor in increasing plant productivity (1,2,6,11). Although, plant density has been studied in some detail as a crop management base practices, the molecular of the plant density effects has been overlooked at both genetic and epigenetic levels (8,9,20). DNA methylation is an epigenetic alteration plays essential role in genome protection, organizing chromatin structure and hence, gene expression. This type of epigenetic modification can be regulated via different mechanisms, nevertheless RNAs-directing methylation is the most common mechanism (3,14,10). In recent years, epigenetic had received considerable attention as an important source of inherited variations, that have been used to improve the overall performance of selected genotypes through the development of breeding methods, as well as their vitak role in tracking environmental impacts at the molecular level (7,16,18). Methods based on epigenetic markers and the diagnosis of gene expression may improve the ability of plant breeders to identify the finest genotype that can tolerate the environmental stresses in general, and plant density in particular (12,13). Production of free radicals is a common feature of stress conditions, and it is known that antioxidants like SODs are the most important defensive tools against such radicals. The SOD responds to the oxidative stress by decomposing free radicals and converting them into hydrogen peroxide (H_2O_2). This process requires certain metal ions (Fe, Cu, Zn and Mn) which acting as SOD's coenzymes (4,15, 19). This study was aimed to detect variations in DNA methylation and FeSOD gene expression in inbred lines and their corresponds diallels under the effect of plant density stress.

MATERIALS AND METHODS

This study was included 15 inbred lines of maize, five of which were selected for half diallel matting according to the second method proposed by Griffing (5). The experiment was conducted during two seasons, Spring and fall 2017 conducted at the field trail station of Department Field Crops -College of Agriculture-University of Anbar. Diallel matting scheme was conducted in Spring season, while in fall season genotypes were compared under two different densities (213333 p. h^{-1} and 13333 p. h^{-1}).

Plant Sample Collection

Fresh plant samples were collected at the end of vegetative growth (about 50 days after planting) for total DNA extraction. Total DNA was extracted with aid of Genomic DNA Mini Kit - Plant (Geneaid Biotech Ltd., South Korea) according to the supplier instructions. Also, fresh maize leaves from all treatments were dipped in trizol solution (600 µl) for RNA extraction by using one-step RT-PCR Kit, (QIAGEN,Hilden, Germany) and supplier instructions was followed.

DNA concentration was ranged between 1.8 -2 measured by using the Nano drop.

Purity of DNA = O.D 260 / O.D = 280 =≥ 1.8

O.D: Optical Density

Three used restriction enzymes EcoR1, Hpall and Mspl were obtained from Promega company (Promega-Madison, Wisconsin-USA). Enzymes were prepared and designed to DNA restriction according to the instructions of the supplier.

Pre-Amplification step

Pre amplification reaction was performed as follows thermal profile: Initial denaturation was at 95ºC for 5 min.; denaturation was at 95ºC for 30 sec.; annealing was at 56ºC for 1 min.; extension and final extension was at 72ºC for 1 and 7 min., respectively. Thye Two adaptors used (Eco and H/M) were with the follows sequence '3-CTCGTAGACTGCGTACC-5' and '3-GATCATGAGTCCTGCT-5', while the Eco Pre-amplification primer and H/M Pre-amplification primer were with follows sequence '3-GACTGCGTACCAATTCA-5' and '3-ATCATGAGTCCTGCTCGG-5'.

Final Amplification step

The following thermal profile used for final amplification step: (Initial denaturation was at 95ºC for 5 min.; denaturation was at 95ºC for 30 sec.; annealing was at 65ºC for 30 sec.; and extension was at 72ºC for 1 min.) for 12 cycle, followed by denaturation set at 95ºC for 5 min. and 30 sec. respectively, annealing was a one

at 56ºC for 30 sec., and extension was at 72ºC for 1 min. for 23 cycle. Final extension was at 60ºC for 30 sec. for one cycle. Two MSAP primers (H/M-1, '3-ATCATGAGTCCTGCTCGGTCG-5' and H/M-2, '3-ATCATGAGTCCTGCTCGGTTG-5') were used to amplify the methylation of susceptible loci.

Electrophoresis

Ten microliters (10 µl) from each amplified product was loaded into the wells. Electrophoresis was performed at a voltage of 5 volt cm^{-1} for 30 min. Agarose gel was placed on UV transilluminator, photographed and documented with gel documentation system. The results of H/M isoschizomers were marked with 0 and 1 numbers to the absence or the presence of a fragment respectively. Binary data (0-1) of MSAP results were analyzed using MSAP package (version 1.1.9).

qPCR conditions

The thermal reaction of gene expression was as follows:

Reverse transcription was at 37ºC, for 15 min., one cycle; Hot-start Activation was at 95 ºC for 10 min. in 1 cycle. Denaturation, Annealing and Extension were at 95ºC, 60ºC and 72ºC for 10, 30 and 30 sec., respectively, and all were run for 40 cycles. The last step of the dissociation was at 60-95ºC in 1 cycle. The expression of FeSOD gene was studied with the aid of two specific primers, forward '3-TTCACTACCAAGGTCTGGACACGA-5' and reverse primers '3-AATAGGCGAGTCGGAATGTCGACA-5'.

After obtaining the (Ct) averages of the gene used under the influence of high and low plant density, the following calculations are made for gene expression:

Y= -3.49 x + 34.33

X= y – 34.33/-3.49

Y= ct

X= log con

** Copy= $10^{\text{Log con}}$

RESULTS AND DISCUSSION

Molecular results of MSAP as affected by plant densities

Results of MSAP molecular analysis (Table 1) using HpaII and MspI restriction enzymes showed that the two enzymes were successful in diagnosing a total of 23 specific loci, Methylation-Susceptible Loci (MSL) was of 22, while only one locus found to be No Methylated Loci (NML). The polymorphism of 13 MSL recorded a percentage of polymorphism around 59%. Shannon's Index rate for MSL was about 0.53. The first primer produced a total of nine restricted loci along with the genotype's genomes under high plant density. Eight (8) of these loci were found to be MSL, against the only one NML. Three (3) of the MSL were polymorphic, scoring a polymorphism percentage of 38%. At the low plant density, the total number of identified sites by the same primer increased to eleven loci indicats low methylation level compared to the highest plant density. Ten of these loci were MSL, and only three out of these ten were in different form, hence the polymorphism percentage reached 30%. The number of NML was limited to one locus that was in different form. Thus, the percentage of polymorphism was complete (100%). The second primer has amplified twelve restricted loci in both densities and all were MSL, but what characterizes the performance of this primer is that the loci were divided equally between poly and mono-morphic (6 to 6) accordingly To the polymorphism percentage rated 50%. The number of polymorphic loci increased to reach nine out of a total of 12 with a final polymorphism percentage of 75%.

Table 1. MSAP results in maize origins and hybrids under plant density effect

Densities	Primer	Loci/Primer	Loci Type		Polymorphic Loci		Polymorphism%		Shannon's Index	
			MSL	NML	MSL	NML	MSL	NML	MSL	NML
High Plant Density (213333 p h^{-1})	H/M1	9	8	1	3	0	38	0	0.43	---
	H/M2	12	12	0	6	0	50	0	0.54	---
Low Plant Density (13333 p h^{-1})	H/M1	11	10	1	3	1	30	100	0.44	---
	H/M2	12	12	0	9	0	75	0	0.49	---
Total		*23*	*22*	*1*	*13*	*0*	*59*	*0*	*0.53*	*---*

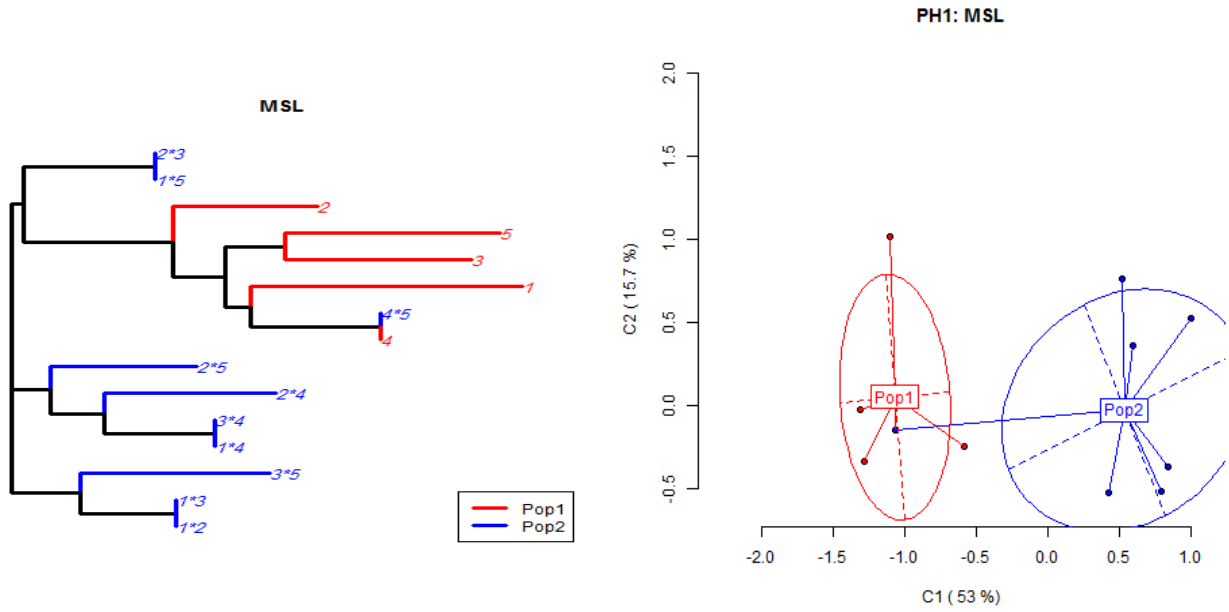
Methylation status in maize genotypes affected by plant density: Five comparisons were settled to describe the four DNA methylation status in MSAP technique (Table 2). The first comparison included the parental inbreds vs. their half-dialleles affected by plant density. The results showed that the percentage of unmethylated state in the inbreds population was higher (0.18%) than in the hybrids population (0.14%). In the hemimethylated state, it was noted that its percentage in the hybrids population was slightly lower than what was detected in their parents population (0.33% and 0.35%, respectively). From the same table, the stress of plant density was approved in the parents via the high level of internal cytosine methylation (0.20%), compared with their F1's (0.16%). In contrast, the full methylation or absence of target was higher in the half diallel hybrids compared to its ancestor parents (0.36% and 0.27%, respectively). The unmethylated state in inbred lines was not affected by the reduction in number of plants in per unit area. It maintained the same percentage as it was in the high plant density (0.18%). Single hybrids response to different plant density was modest as it showed a percentage of 0.15%. The percentage of hemimethylated state in the diallel hybrids was 0.44%, compared with a lower percentage recorded in the parents population (0.30%). Paternal breeds returned to show a higher percentage of internal cytosine methylation (0.19%), compared to their F1s hybrids (0.13%), but both were lower in low plant density against high density.The exposure of maize plants to density stress resulted in a marked variation in their genetic and epigenetic performance, which in turn, reflected in phenotypic variation. Full methylation or absence of target state at high plant density was quite different from that found in the low plant density. This stat was decreased from 0.33% in inberds population to 0.27% in their descended hybrids. To achieve a better understanding, methylation status was estimated for each population at the two different plant densities. The results indicated that the percentage of unmethylated state reached 0.18% at the higher density compared with 0.16% at the lowest density. Another methylation status (hemimethylated state) differed slightly (0.32% and 0.30%) in the inbred lines population under highest and lowest densities, respectively. Such a close epigenetic performance still existed in the methylation state of internal cytosine at the two densities (0.18% and 0.19%, respectively), furthermore the percentage of full methylation or absence of the target which reached 0.34% and 0.33% at highes and lowes number of plants per unit area. The other comparison showed a clear response of half diallel hybrids to the different densities. These hybrids showed higher percentages at lowes plant density for both, unmethylated and hemimethylated states (0.18% and 0.43%, respectively), compared to the modest percentages recorded at the highest density (0.17% and 0%. 29%, respectively). Conversely, the level of internal cytosine methylation and full methylation states related directly to the number of competitive plants as both reached (0.14% and 0.40%) in highest plant density and (0.12% and 0.26%) in lowest density.

Table 2. Comparison of the Methylation Status in Parental and their Half Diallel Populations Affected by Plant Density

Compared Populations		Methylation Status %			
		Unmethylated	Hemimethylated	Internal cytosine methylation	Full methylation or absence of target
		HPA+/MSP+	HPA+/MSP-	HPA-/MSP+	HPA-/MSP-
Parents vs. Hybrids (High Density)	Parents	0.18	0.35	0.20	0.27
	Hybrids	0.14	0.33	0.16	0.36
Parents vs. Hybrids (Low Density)	Parents	0.18	0.30	0.19	0.33
	Hybrids	0.15	0.44	0.13	0.27
Parents vs. Parents	High Density	0.16	0.32	0.18	0.34
	Low Density	0.18	0.30	0.19	0.33
Hybrids vs. Hybrids	High Density	0.17	0.29	0.14	0.40
	Low Density	0.18	0.43	0.12	0.26

Principal Coordinates Analysis (PCoA) and phyloepigenetic analysis of MSAP results affected by plant density

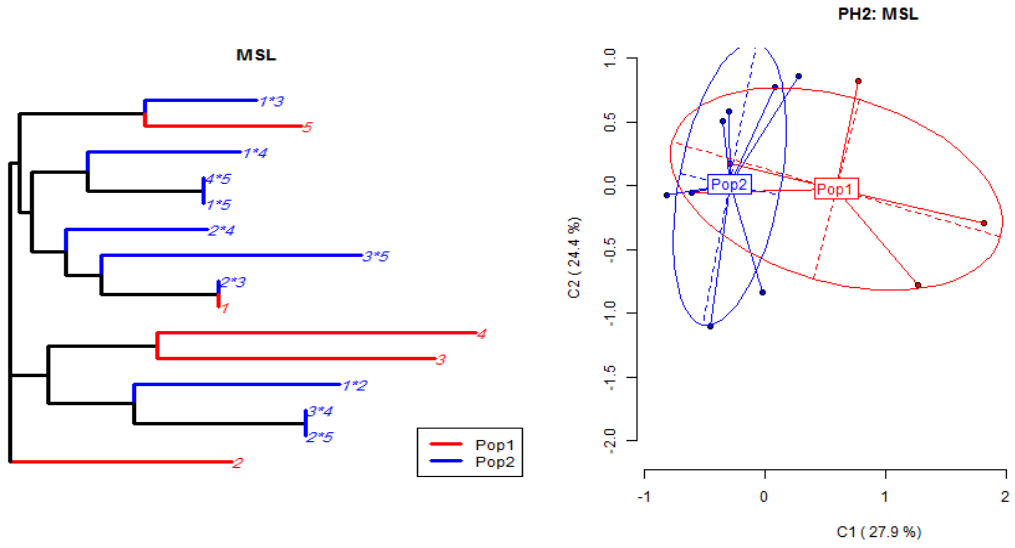
The principal coordinates analysis of MSAP resulted in the parental population (Pop.1) and their half-dialle hybrids (Pop.2), under high plant density, showed that the highest variation (53%) was recorded at the first coordinate (C1), against lower variation (15.7%) at the second coordinate (C2) (Fig1. A). It seems that both populations tend to form a separate group and only single interferance was detected between hybrid 4×5 which had affected by the specific pattern of DNA methylation showed by it's own parental inbreed (4). The overall performance was less homogenous in hybrids compared to the ancestor inbred lines. The results of cluster analysis (Fig1. B) indicated that the parents were characterized by a special pattern of DNA methylation and marked with individual performance. In epigenetic terms, parents 1 and 5 were the most divergent in response to plant density stress.



(A) (B)

Fig1, Principal coordinates analysis (PCoA) (A) ,and Hierarchical clustering according to Nearest neighbor method (B) for DNA MSLs in the parents (Pop1) &maize half-diallel hybrids (Pop2) under the effect of high plant density (213333 H-1).

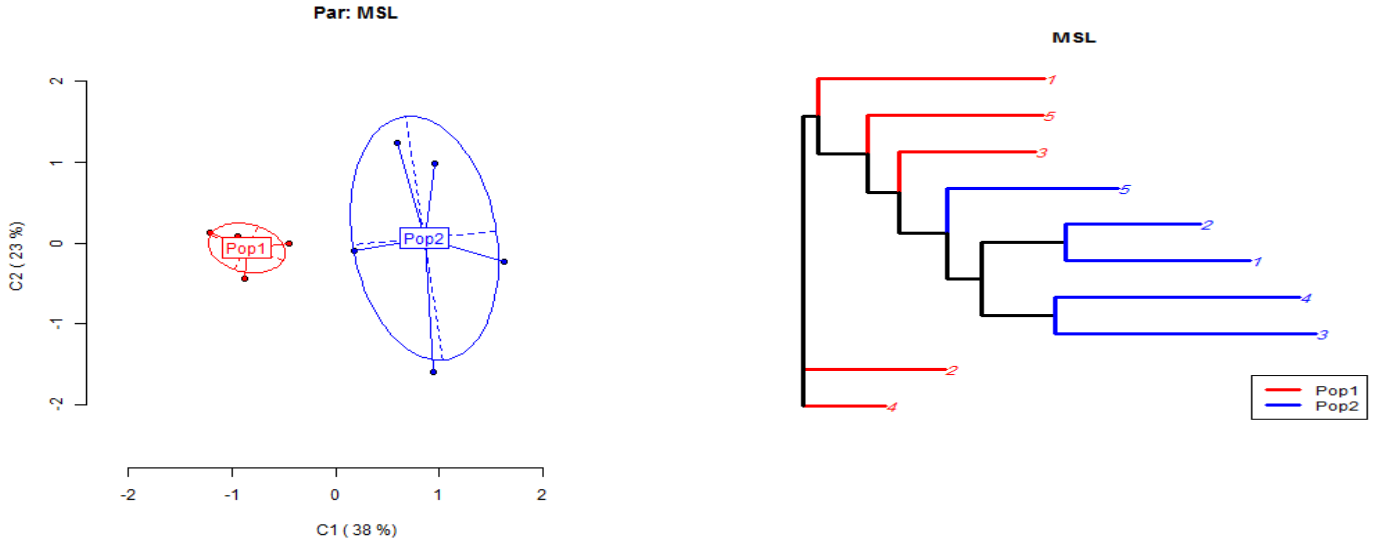
Reduction of plant density (Fig2. A and B) that gave adequate growing space, resulted in more homogenous pattern of DNA methylation to the extent that the horizontal boundary between the two populations disappeared. This was confirmed by the value of coordinate C1, which reached 27.9%, while the value of the second coordinate (C2) reached 24.5%. This alteration in the epigenetic performance in response to different plant densities rled to different "phyloepigenetic" tree, in which inbred a 2 became the most epigenetically divergent to hybrid 1×3, while inbred 1 showed high level of epigenetic similarity with hybrid 2×3.



(A) (B)

Fig2, Principal coordinates analysis (PCoA) (A), and Hi Hierarchical clustering according to Nearest neighbor method (B) for DNA MSLs in the parents (Pop1) and maize half-diallel hybrids (Pop2) under the effect of low plant density (13333 plant H-1)

Effect of plant densities were approved as the paternal inbreds formed two distincted groups based on the plant density. These inbreds showed more homogeneous epigenetic performance at higher density compared to low density. The value of the first coordinate (C1) was 38%, while it was 23% at the second coordinate (C2). Inbeds 1 and 4 were the most divergant in respect of epigenetic structure at high density, whereas inbreds 3 and 5 had this role at the lowest plant density.

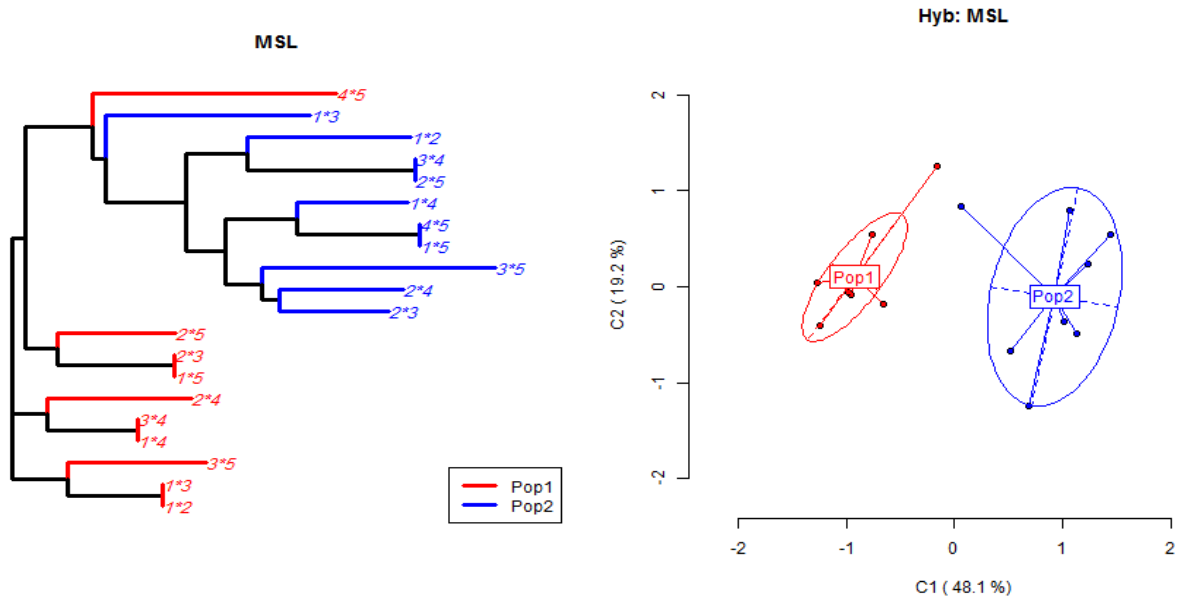


(A) (B)

Fig3, Principal coordinates analysis (PCoA) (A) ,and Hi Hierarchical clustering according to Nearest neighbor method (B) for DNA MSLs in the parents ofmaize (Pop1) under the effect of high plant density (213333 plant H-1) and low plant density (Pop2), (13333 plant H-1)

Distribution of Methylation-Sucetable Loci (MSL) in half diallel hybrids confirmed the results of the major effects of planting density stress in the epigenetic structure of maize genome. The epigenetic performance of the hybrids at the high plant density can be easly distinguished by their performance at the lowest plant density, hence the two separated

groups did not interfere only in one hybrid (4×5) that tends to belong to the other density recital. The epigenetic variation in hybrid population under the two densities was higher at the first coordinate (48.1%) than the second coordinate (19.2%). Cluster analysis of hybrids MSL results under the two plant densities (Fig4. B) confirmed the epigenetic distinctness of 4×5 and 3×5 hybrids, under high and low plant density, respectively.



(A) (B)

Fig4, Principal coordinates analysis (PCoA) (A) ,and Hi Hierarchical clustering according to Nearest neighbor method (B) for DNA MSLs in the half-diallel hybrids of maize (Pop1) under the effect of high plant density (213333 plant H-1) and low plant density (Pop2), (13333 plant H-1)

Fe-SOD Gene Expression

Estimation of FeSOD gene expression (Table 3) showed a significant difference among studied genotypes (parents vs. half-diallel hybrids) and plant densities (high (213333 plant h^{-1}) and low (13333 plant h^{-1}). The exceptional performance of inbred 3 at the higher plant density can be observed as it achieved the maximum number of targeted gene copies (5505.7). When plants have enough space to grow and were less competted at the lower density, the same parent gave the highest number of FeSOD copies (1704.7), with a gap of 3801 copy between the two densities. As expected, the growing environment has a key role in the genetic performance of maize genotypes but with different levels, hence, genetic-environment interaction is highlighted. Some genotypes respond positively or negatively to plant density stress, meanwhile some other genotypes showed relatively consistent performance at the different densities, as parent 1, that still has the lowest FeSOD gene expression in its cells at both high and low densities (1375.8 and 1569.5 copy, respectively). The importance of such genotypes is shown to be epigenetically stable under stress conditions, which may be associated with a distinctly superior genetic pattern that ensures greater regulation of gene expression. The parents passed the existed variation in FeSOD gene expression to their F1 hybrids. The response of hybrids to the increasing number of plants in the unit area was varied. However, hybrid 1X3 took the lead in number of copies of antioxidant FeSOD gene at the higher plant density (21098.6 copy) with a gap of 17488.4 copy compared to the modest number of copies (17488.4 copies) gained by the same hybrid at the lowest plant density. On the other hand, hybrids 2×5 and 4×5 revealed negative response to FeSOD gene expression due to the decreased planting density, with a gap of 1000

and 4508 copy between the maximum and minimum planting densities, respectively.

Table 3, Expression level of Fe-SOD in parental breeds (1-5) and half-diallel hybrids (1×2-4×5) under plant density (high and low) in maize

Low Plant Density (13333 plant h^{-1})		High Plant Density (213333 plant h^{-1})	
FeSOD Expression	Genotype	FeSOD Expression	Genotype
1569.5	1	1375.8	1
2486.0	2	1828.9	2
1704.7	3	5505.7	3
6000.4	4	5089.1	4
8317.6	5	1424.1	5
2821.3	1×2	12528.5	1×2
3610.2	1×3	21098.6	1×3
4412.3	1×4	3187.9	1×4
3096.8	1×5	2910.2	1×5
2292.9	2×3	3668.6	2×3
4639.4	2×4	2739.1	2×4
2755.7	2×5	1769.4	2×5
5145.3	3×4	844.8	3×4
3959.5	3×5	2845.8	3×5
6862.1	4×5	2354.9	4×5

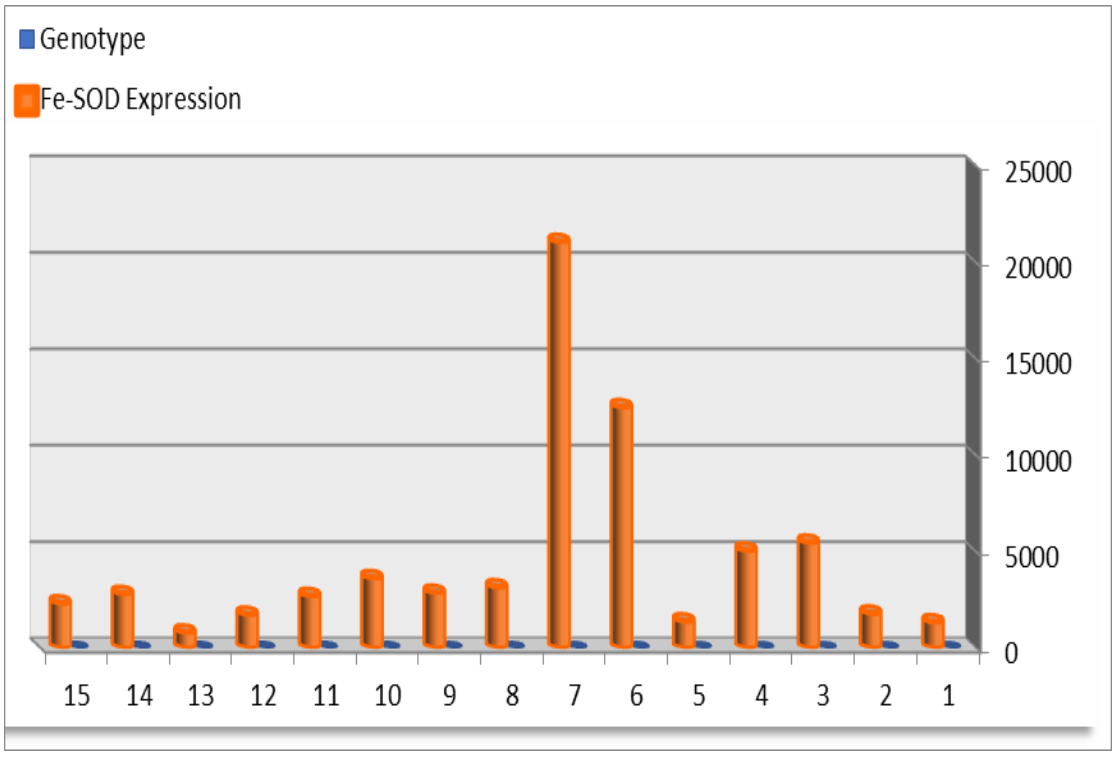


Fig5. Level of Fe-SOD expression in the parental breeds (1-5) and their half-diallel hybrids (6-15) under the influence of the high plant density (213333 plants H-1) in the maize

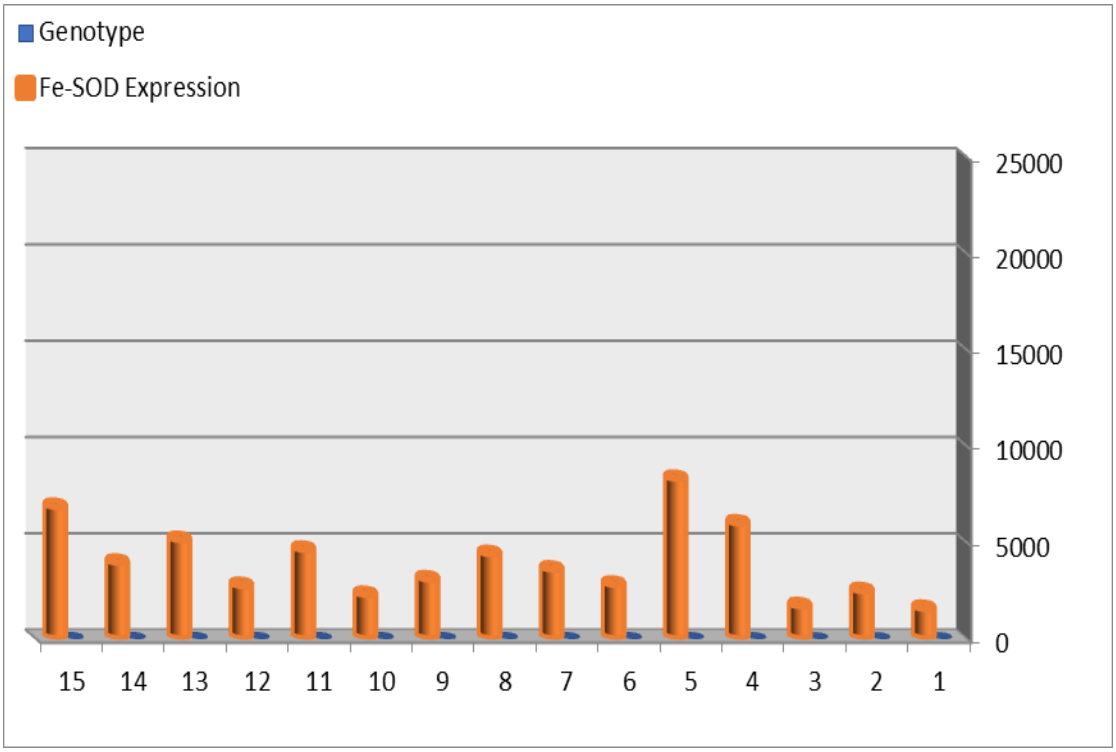


Fig6. Level of Fe-SOD expression in the parental breeds (1-5) and their half-diallel hybrids (6-15) under the influence of the low plant density (13333 plants H-1) in the maize

REFERENCES

1. Aldaoodi, A. H.R., Kh. Kh, A. Jubouri ., M. I. M. Alokaidi. 2015. The response of three hybrids of maize (*Zea mays* L.) for plant density and nitrogen fertilizer. Diyala Journal of Agricultural Sciences. 7 (1): 133 147

2. Al-Khazali, H. A., M, M. Al-Sahookie., F, Y. Bektaşh. Genetic Variations of some characters of maize under different plant densities. Journal of Iraqi Agricultural Sciences. 44 (3): 300 308

3. Candaele, J., K. Demuynck, D. Mosoti, G. Beemster, D.Inzé, and H. Nelissen, 2014. Differential methylation during maize leaf growth targets developmentally regulated genes. Plant Physiology, 164(3): 1350-1364

4. Feng, K., J. Yu ,Y. Cheng, M. Ruan, R. Wang, Q. Ye, G. Zhou, Z. Li, Z. Yao, Y. Yang, Q. Zheng, and H. Wan. 2016. The SOD gene family in tomato: Identification, phylogenetic relationships, and expression patterns. Front. Plant Sci., 7:1279. doi: 10.3389/fpls.2016.01279

5. Griffing, B, 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Australian J. Biol. Sci., 9: 463–496

6. Guo, J., Z. Chen, Z. Liu, B. Wang, W. Song, W. Li, and J. Lai. 2011. Identification of genetic factors affecting plant density response through QTL mapping of yield component traits in maize (*Zea mays* L.). Euphytica, 182(3): 409

7. Gutzat, R. and O. M. Scheid. 2012. Epigenetic responses to stress: Triple defense? Curr. Opin. Plant Biol., 15: 568–573

8. Hamdan, M. I.; F, Y. Bektash. 2014. Extracting synthetic varieties from mixing several maize inbreds using plant densities. Anbar Journal of Agricultural Sciences. Vol. 12 (2): 251 263

9. Ku, L., Z. Ren, X. Chen, Y. Shi, J. Qi, H. Su, and J. Zhou. 2016. Genetic analysis of leaf morphology underlying the plant density response by QTL mapping in maize (*Zea mays* L.). Molecular breeding, 36(5): 63

10. Madzima, T. F., J. Huang, and McGinnis, K. M. 2014. Chromatin structure and gene expression changes associated with loss of MOP1 activity in *Zea mays* L. , Epigenetics, 9(7): 1047-1059

11. Manzvera, T. 2017. Effects of population density and water stress on morphology of tropical maize genotypes. J. Crop Res. Fert., 1: 1-9

12. Mao, H., L. Yu, Z. Li, H. Liu, and R. Han. 2016. Molecular evolution and gene expression differences within the HD-Zip transcription factor family of *Zea mays* L. Genetica, 144(2): 243-257

13. Pande, A. and S. Arora. 2017. Molecular strategies for development of abiotic stress tolerance in plants. Cell & Cellular Life Sciences Journal, 2(2): 000112

14. Saze, H., K. Tsugane, T. Kanno, and T. Nishimura. 2012. DNA methylation in plants: relationship to small RNAs and histone modifications, and functions in transposon inactivation. Plant and Cell Physiology, 53(5): 766-784

15. Shiriga, K., R. K. Sharma, S. Kumar, K. Yadav, F. Hossain, and N. Thirunavukkarasu. 2014. Expression pattern of superoxide dismutase under drought stress in maize. Int J Innovative Res SciEngTechnol, 3(4): 11333-7.

16. 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

17. Sunkar, R., Y-F. Li and G. Jagadeeswaran. 2012. Functions of microRNAs in plant stress responses. Trends Plant Sci., 17: 196–203

18. Turunen, T.A., M. A. Väänänen and S. Ylä-Herttuala. 2018. Epigenomics. In: R. Vasan and D. Sawyer. The Encyclopedia of Cardiovascular Research and Medicine, 2: 258-265

19 Wang, W., X. Xiaopei, F. Deng, R. Yuan and F. Shen. 2017. Genome-wide characterization and expression analyses of superoxide dismutase (SOD) genes in *Gossypium hirsutum*. BMC Genomics, 18:376. doi: 10.1186/s12864-017-3768-5

20. Wuhaib, K. M., B.H. Hadi, W.A. Hassan. 2017. Estimation of genetic parameters in sorghum under the effect of populations and planting seasons. Journal of Agricultural Science of Iraqi, 48(2): 551-563.