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GENETIC POLYMORPHISM OF SEED STORAGE PROTEIN AND ISOZYMES IN MAIZE

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ABSTRACT: Electrophoretic analyses of storage proteins and isozymes were used to identify and differentiate between six inbred lines of maize and their F_1 hybrids. Twenty two alcohol – soluble protein bands were detected in all genotypes. The bands were different in their intensities. Eight bands were common in all inbred lines and hybrids. The F_1 hybrids showed more bands than their respective parents. In additon, the esterase and peroxidase isozyme systems studied, manifested ten bands that were common in all inbred lines and hybrids. Isozyme pattern were satisfactory in identifying the 21 maize genotypes. The cluster analysis describing the relationships among the 21 maize genotypes exhibited only two clusters based on the results of protein and isozymes pattern. The data are useful for varietal identification.

Key words: **Maize, storage proteins, isozymes, esterase, peroxides, cluster analysis.**

INTRODUCTION

The major storage protein fraction in maize consists of a group of alcohol- soluble proteins (prolamines) commonly called "zein", which is represented more than 50% of total protein in endosperm. Zein proteins are well known for their poor nutritional quality resulting from negligible contents of lysine and tryptophan.

The protein patterns contained from eight to twelve bands with a high level of heterogeneity among the varieties of maize and among the lines within a variety. Furthermore, the protein patterns showed

differences between hybrids and maternal types. (Ohms, 1985).

Abdel- Tawab *et al.*, (1989) and Kamarova *et al.*, (1992) found that the electophoretic analysis of endosperm protein and isozymes were effective in the identification of inbred and crosses, and as biochemical genetic markers associated with hybrid vigor and combining ability in maize.

Also, Abdel- Tawab *et al.*, (1993) fingerprinted twelve inbred lines of maize by electrophoretic fractions of grain protein and isozymes and found measurable genetic variations among these inbreds and distinguishing twelve class patterns, unique class for each line.

Variation was found in major fractions and minor bands which are directly related to the genetic background of genotypes that can be used to certify the genetic makeup of wild, cultivated or newly derived maize (Gorinstein *et al*, 1999).

The present work aimed to identify and assess the genetic polymorphism among maize genotypes based on differences in storage protein and isozymes banding patterns.

MATERIAL AND METHODS

Six yellow maize inbred lines were used in this study. These lines were 6 (P_1), 8 (P_2), 19 (P_3), 21 (P_4), 22 (P_5) and 23 (P_6).The inbred lines were kindly supplied by Maize Research Section, Agriculture Research Center, Giza, Egypt.

In the season of 2001, the inbred line grains were planted and a half diallel cross, among the six parents were done. In the next season 2002 the six parental inbreds and 15 F_1 hybrid were planted at Kaafr EL- Hamam Village, Zagazig, Sharkia Governorate, in a randomized complete blocks design with three replications. Each replicate contained 21 rows, one row for each genotype, parent or F1. Each row was 3.5 m long and contained 10 plants. One week-old seedlings were taken for isozymes analysis using elecrophoretic technique according to (Bradford 1976). The procedures of Tanksely and Rick (1980) for peroxidase detection and of Jonathan and Wendel (1990) for esterase detection .For seed storage protein analysis, parental of as well as F_1 grains, the electrophoretic were applied

technique according to Studier (1973). To study clustering pattern among maize genotypes based on band differences banding patterns , data generated from peroxidase , esterase and zein protein variations were introduced to SPSS package program. Genetic distance calculated as a Euclidean matrix distances, was computed between all pairs of populations. Hierarchical clustering procedure was applied using Ward's method as outlined by Anderberg (1973) and developed by Hair *et al.* (1987). The biochemical analyses were done at the Biochemical Laboratary, Genetics Dept., Ain Shams Univ.

RESULTS AND DISCUSSION

1. Seed storage protein electrophoretic pattern :

The electrophoretic patterns for alcohol soluble protein (zein) of 21 maize genotypes are illustrated in Figure (1). Data showed that approximately 22 bands were detected with different molecular weights ranged between 145.75 and 17.54 KDa. These bands were present in some genotypes and absent in the other. The electrophoretic bands showed a wide variation in their molecular weight. Common band patterns in all genotypes (monomorphic) were observed. While, 13 bands were not detected in some genotypes (polymorphic). The band at molecular weight of 130.25 KDa was a common bands in all genotypes except the hybrids P_3 x P_4 and P_3x P_6.The band at molecular weight of 120.95 KDa was a common band in all genotypes except P_1, P_2 and the hybrid P_3x P_6.The bands at molecular weights of 104.79, 91.8 and 68.93 KDa were not distinguished in all inbred lines but in some hybrids.The bands at molecular weights of 79.68 and 61.36 KDa were distinguished only in the hybrids P_3 xP_5 and P_2 x P_3 .However, the band at molecular weight of 57.17 KDa was only detected in P_1 .Our results are in agreement with those of Komarova *et al.*, (1992) and Abdel- Tawab *et al.*, (1993) who found variations in zein electrophoretic patterns among maize genotypes.

2. Isozymes electrophoretic patterns:

A- peroxidase:

The zymogram of isoperoxidase banding pattern in seedlings of six maize parents and their 15 F_1's

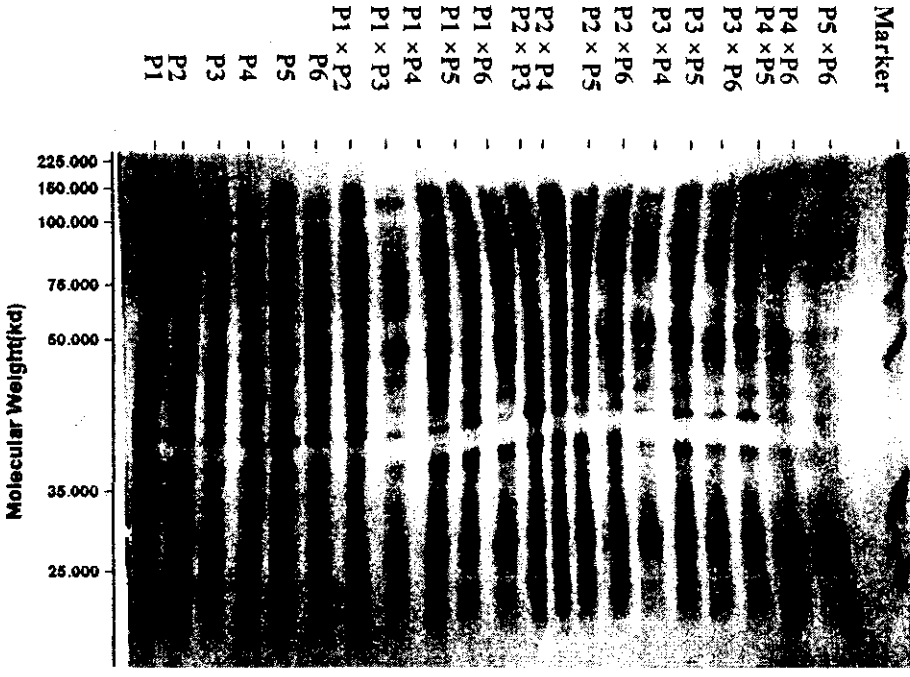


Figure (1): Electrophoretic pattern of zein in seed storage proteins extracted of parents and F_1 hybrids of maize .

showed 10 peroxidase anodal bands (Figures 2 and 3).

The fast migrating band, at the position of 6.0 cm anodal to the origin, appeared to be present in all parents except P_2 and some F_1's, suggesting that this band might be controlled by the presence versus absence alleles. The variants at the positions 0.5, 1.1, 2.6, 5.0 and 5.4 cm anodal to the origin were found to be common bands in all parents and F_1's, i.e. universal bands. The variants at the positions 4.4, 5.6, 3.3 and 4.0 cm anodal to the origin were expressed in most parents and F_1's.

These results might reflect a case of variations in isoperoxidase banding patterns among maize genotypes. In additions, these electrophoretic bands showed wide variation in their intensities, ranging from faint to dark, reflecting different peroxidase activities in the seedling tissues of these genotypes.

In this regard, Revin and Rotar (1981) found marked differences in isoperoxidease patterns between normal forms and their counterparts at all maize in germination stages. Also, Geetha and Jayaraman (1998) stated that all the 12 genotypes of maize had the same banding patterns at loci 1 and 6.

B- Esterase:

The zymogram of isoesterase banding pattern in seedlings in six maize parents and their 15 F_1's showed 10 esterase anodal bands (Figures 4 and 5).

The fast migrating band, at the position of 6.0 cm anodal to the origin, appeared to be present in all genotypes except P_6 and hybrids (P_1xP_4,P_1 x P_6, P_2xP_3 and P_3 x P_4). This result might suggest that this band might be controlled by the presence versus absence alleles. The variants at the positions 0.6, 4.7, 5.1, 5.5 and 5.6 cm anodal to the origin were found to be have as common band in all parents and F_1 hybrids, i.e. universal bands. The variants at the positions 3.4. 1.5, 4.2 and 2.5 cm anodal to the origin were expressed in most parents and F_1's.

These results might reflect a case of variations in isoesterase banding patterns among maize genotypes. In additions, the electrophoretic bands showed wide variations in their intensities, ranging from faint to dark, reflecting different esterase activities in the seedling tissues of these genotypes.

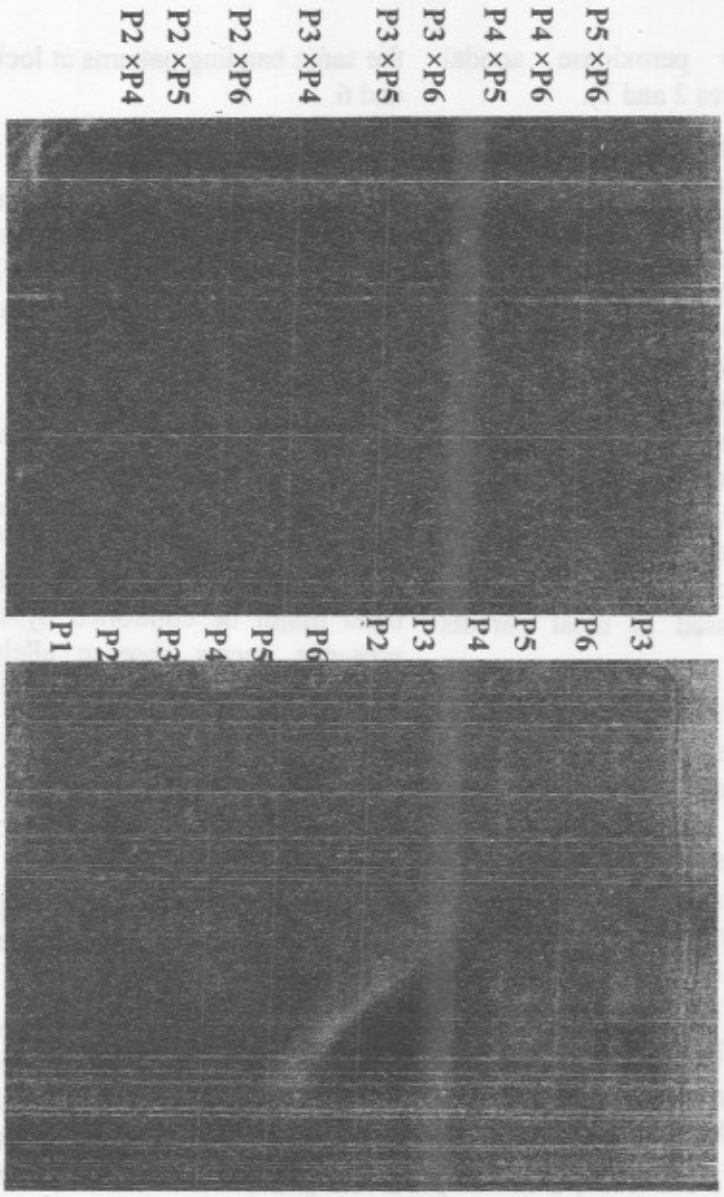


Figure (2) : Polyacrylamide gels for peroxidase isozymes in seedling tissue extracts of six maize parents and their F_1 hybrids.

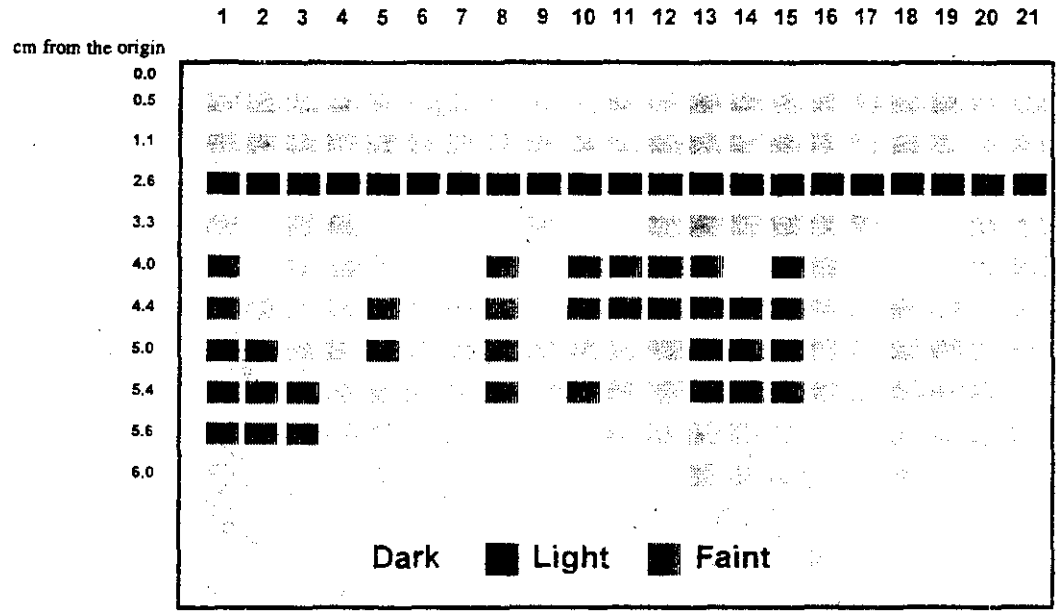


Figure (3) : Zymogram peroxidase isozymes pattern of 21 maize genotypes

(1=P1, 2=P2, 3=P3, 4=P4, 5=P5, 6=P6, 7=P1XP2, 8=P1XP3, 9=P1XP4, 10=P1XP5, 11=P1XP6, 12=P2XP3, 13=P2XP4, 14=P2XP5, 15=P2XP6, 16=P3XP4, 17=P3XP5, 18=P3XP6, 19=P4XP5, 20=P4XP6, 21=P5XP6)

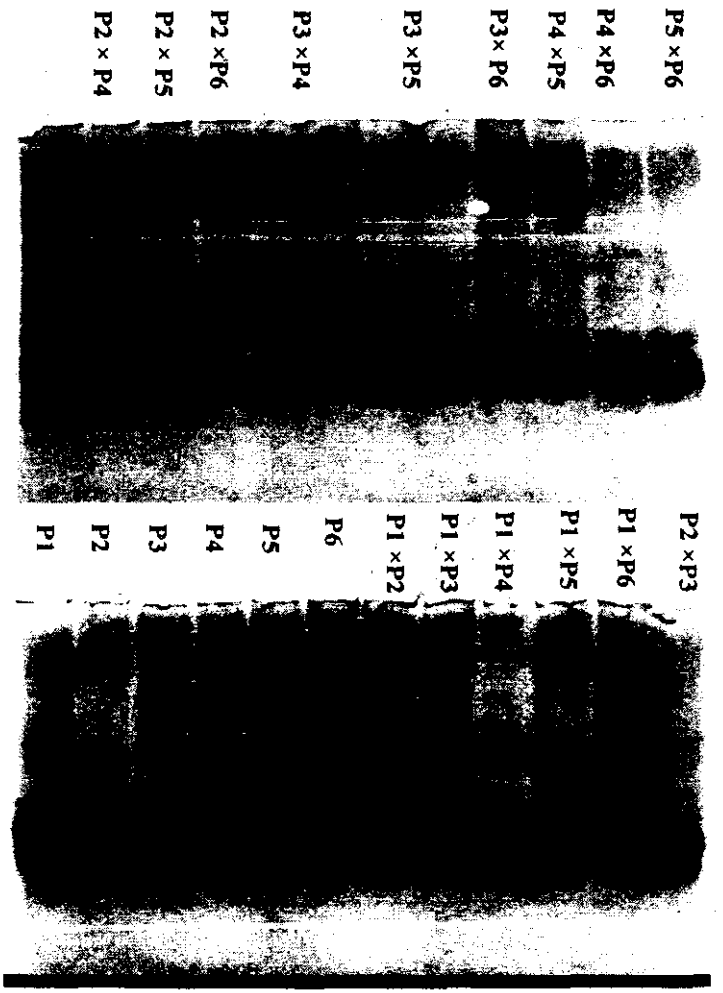


Figure (4) : Polyacrylamide gels for esterase isozymes in seedling tissue extracts of six maize parents and their F_1 hybrids.

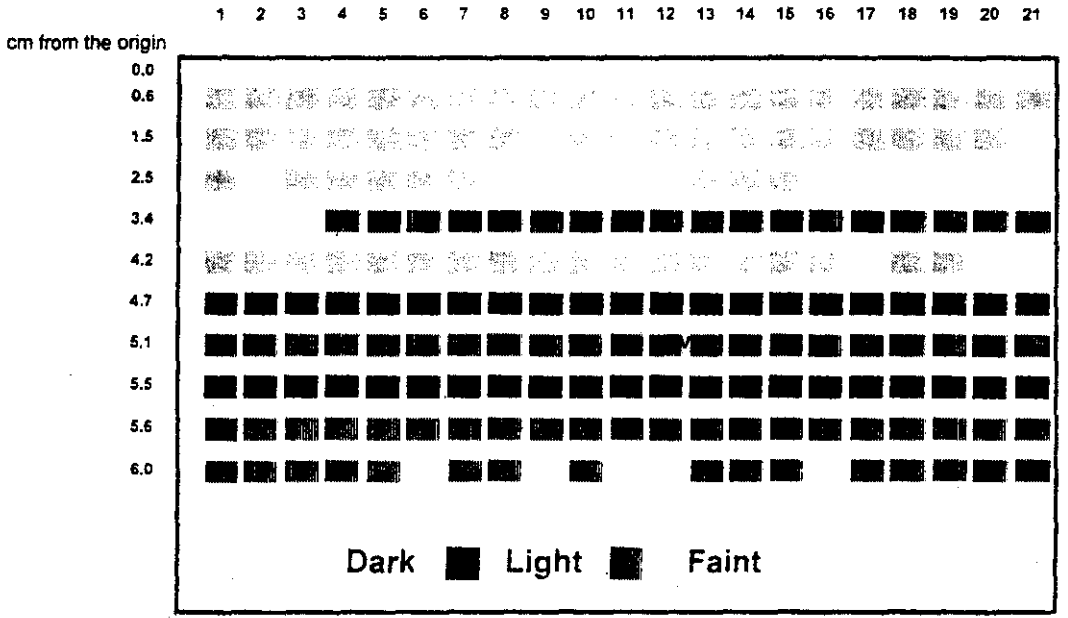


Figure (5) : Zymogram esterase isozymes pattern of 21 maize genotypes

(1=P1, 2=P2, 3=P3, 4=P4, 5=P5, 6=P6, 7=P1XP2, 8=P1XP3, 9=P1XP4, 10=P1XP5

11=P1XP6, 12=P2XP3, 13=P2XP4, 14=P2XP5, 15=P2XP6, 16=P3XP4, 17=P3XP5, 18=P3XP6, 19=P4XP5, 20=P4XP6, 21=P5XP6)

In this regard, Heidrich – Sobrinho and Coreerro (1975) showed variation of four esterase loci Est_1, Est_2, Est_3 and Est_4 which are controlled by slow and fast electrophoretic bands. Zhang and Tang (1983) found that in esterase zymograms in maize mutants, only one band was identical to that of the parental band, in addition to three heterozygous bands. Rao *et al.*, (1997) found that specific fast-migrating bands were absent at the growth stages and the number of bands varied by the different stages.

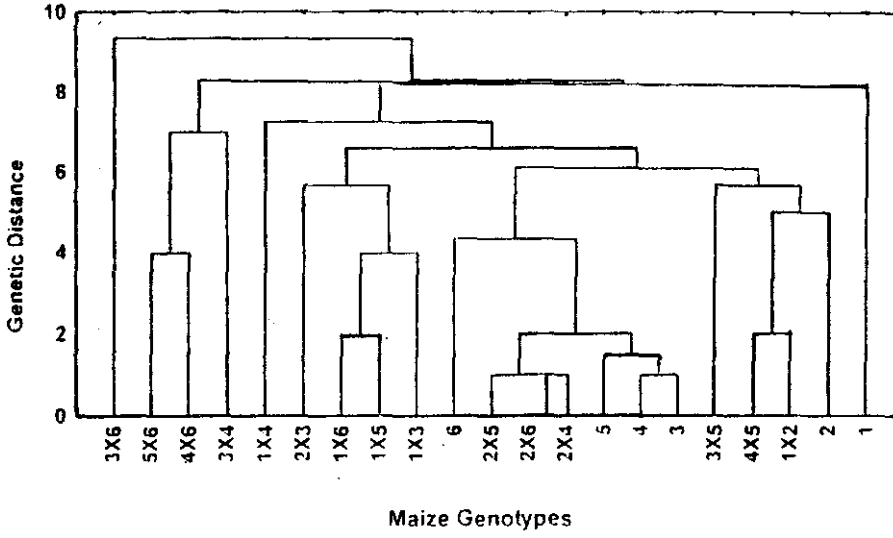
3- **Cluster analysis of maize genotypes based on their peroxidase, esterase and zein protein banding patterns .**

The presence/ absence matrix of peroxidase, esterase and zein protein banding patterns found in studied maize were uesd to generate a measure of genetic distances among the 21 maize populations (parents and hybrids). The index of genetic dissimilarity was calculated pairwise distance. Genetic distance calculated as the total number of band diffrrences, between maize population, are presented in Table (1). Genetic distances were mostly small with the exception of those between a few pairs of poulations .This suggested that there was not enough genetic variability in their peroxidase , esterase and zein protein banding patterns to set any genotype a part from the others . Similar findings were reported by Labuschagne *et al.*, (2000) who found that genetic distances between clusters were small. Thus, the similarity between studied maize could be ascribed to maize populations sharing as a common ancestral population and/or adaptation to similar environmental conditions. The highest magnitude of Euclidean genetic distances among genotypes was observed between $P_3 \times P_6$ and $P_5 \times P_6$, $P_2 \times P_3$ and $P_3 \times P_6$, while the minimum distance was found between $P_2 \times P_4$ and $P_2 \times P_6$, but the most similar populations were $P_1 \times P_2$ and $P_3 \times P_5$, P_3 and P_4 , $P_2 \times P_4$, $P_2 \times P_5$ and $P_2 \times P_6$, $P_1 \times P_5$ and $P_1 \times P_6$, $P_4 \times P_6$ and $P_5 \times P_6$. The dendrogram produced from genetic distances between maize genotypes indicated that considerable genetic divergence was induced by hybridization and that F_1 hybrids were widely dispersed from their parents. These data were shown in figure (6). Based on the extent of relative dissimilarity among genotypes based on their peroxidase, esterase and zein protein banding patterns,

Table (1): Euclidean distances among maize genotypes based on their peroxidase, esterase and zein protein banding patterns.

Genotypes	1	2	3	4	5	6	1x2	1x3	1x4	1x5	1x6	2x3	2x4	2x5	2x6	3x4	3x5	3x6	4x5	4x6
2	6.0																			
3	4.0	6.0																		
4	5.0	7.0	1.0																	
5	6.0	6.0	2.0	1.0																
6	9.0	7.0	5.0	4.0	3.0															
1x2	9.0	5.0	5.0	4.0	3.0	4.0														
1x3	8.0	8.0	6.0	5.0	6.0	9.0	5.0													
1x4	12.0	10.0	8.0	7.0	8.0	7.0	9.0	8.0												
1x5	9.0	7.0	7.0	6.0	5.0	8.0	6.0	3.0	7.0											
1x6	11.0	9.0	9.0	8.0	7.0	8.0	8.0	5.0	7.0	2.0										
2x3	11.0	9.0	7.0	6.0	7.0	8.0	8.0	5.0	7.0	6.0	6.0									
2x4	6.0	8.0	2.0	1.0	2.0	5.0	5.0	4.0	6.0	5.0	7.0	5.0								
2x5	7.0	7.0	3.0	2.0	3.0	4.0	4.0	5.0	5.0	6.0	8.0	6.0	1.0							
2x6	6.0	8.0	2.0	1.0	2.0	5.0	5.0	4.0	6.0	5.0	7.0	5.0	0.0	1.0						
3x4	8.0	8.0	8.0	7.0	8.0	9.0	9.0	6.0	10.0	7.0	7.0	7.0	8.0	9.0	8.0					
3x5	11.0	7.0	7.0	6.0	7.0	8.0	6.0	7.0	7.0	6.0	8.0	8.0	7.0	6.0	7.0	9.0				
3x6	10.0	6.0	10.0	9.0	8.0	9.0	7.0	10.0	10.0	9.0	11.0	13.0	10.0	9.0	10.0	10.0	9.0			
4x5	11.0	5.0	7.0	6.0	5.0	6.0	2.0	5.0	7.0	4.0	6.0	8.0	7.0	6.0	7.0	9.0	4.0	5.0		
4x6	9.0	7.0	7.0	6.0	7.0	8.0	8.0	7.0	11.0	8.0	10.0	8.0	7.0	8.0	7.0	7.0	6.0	9.0	8.0	
5x6	9.0	9.0	9.0	8.0	9.0	10.0	10.0	7.0	11.0	8.0	8.0	10.0	9.0	10.0	9.0	7.0	8.0	13.0	10.0	4.0

Figure (6) : Linkage dendrogram for twenty one maize genotypes based on their peroxidase , esterase and zein protein banding patterns .



the twenty one maize populatoins were grouped into twelve clusters . Cut off point at 5 dissimilarity points (Euclidean distances) was fixed as minimum dissimilarity. Cluster I consisted of one parent (P1); P_4xP_5, P_1xP_2 and P_2 in cluster II; P_3xP_5 in cluster III; P_3, P_4, P_5,P_2xP_4, P_2xP_5 and P_2xP_6 in cluster IV; P6 in cluster V; P_1xP_3, P_1xP_5 and P_1xP_6 in cluster VI; P_2xP_3 in cluster VII; P_1xP_4 in cluster VIII; P_3xP_4 in cluster IX ; P_4xP_6 and P_5xP_6 in cluster X; P_3xP_6 in cluster XI. The Euclidean genetic distances indicated that genotypes in cluster II were the most divergent from genotypes in cluster I. The parents were distributed over three clusters.

The clustering of genotypes using their peroxidase, esterase and zein protein banding patterns proved to be in accordance with their breeding origin. Therefore, it was not surprising that the two maize populations P_3 and P_4, $P_2\times P_4$, $P_2\times P_5$ and $P_2\times P_6$ are segregated in one small cluster. In this regard, Vierling and Nguyen (1992) and Jamal-AL-Shabi (1998) found that the similarity indices were ranged from 0.32 to 0.62 between six wild wheat genotypes, while ranged from 0.41to 0.81 between eight genotypes of cultivated diploid wheats.

REFERENCES

Abdel- Tawab, F.M.; E.M Fahmy; M.A. Rashaed and M.H. Abou-Deif (1989). Protein and isozyme polymorphism as related to heterosis and combing ability in maize. Egypt. J. Genet. Cytology.18, 203- 217.

Abdel- Tawab, F. M.; M.A. Rashed; I. M. Fahmy and A. Baheidin (1993). Electrophoretic characterization of twelve inbred lines of maize (*Zea mays L.*). Annal Agric. Sci, Fac Agric A:n shams Univ., Cairo, 2,417. 427.

Anderberg, M. R. (1973). Cluster Analysis for Application . Academic press, New York.

Bradford, M. M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248 – 254..

Geetha, K. and N. Jayaraman (1998). Genetic polymorphism of maize peroxidases in twelve maize (*Zea mays*) genotypes.

Agricultural- science – Digest – Karnal. 18: 2.14143-144;7 ref.

Gorinstein- S; No. Jaramillo; OJ. Medina; WA. Rogriques; GA. Tosello; O. Pardes- Lopez (1999). Evaluation of some cereals, plants and tubers through protein composition. J. Protein – chemistry 18: 6, 687-693.

Hair, J.S.; Anderson and R.L. Tathem (1987). Multivariate Data Analysis with Readings. Macmillan publi.Co., New York.

Heidrich-sobrinho, E; and A. R. Coreerro (1975). Codiminant isoenzymic alleles as markers of genetic diversity correlated with riterosis in maize (*zea mays L.*) Theor. and Appl. Genet. 46: 197- 199.

Jamal H. M. AL-Shabi (1998). Biochemical and molecular genetic fingerprints for some wheat and wheat- related cultivars. J. Agric. Ain Shams univ., Egypt 12:180- 193.

Jonathan, F.W. and N. F. Wendel (1990). Visualization and Interpretation of Plant Isozymes in Plant Biology (D. E. Soltis and P.S. Soltis Eds), London Champan and Hall, pp.45.

Kamarova, G.;A. Rotar and V. Fudenko (1992). Use of zein electoctophoresis method in maize breeding and seed production in Moldavia Maize. Abs, 8 (8), 2710.

Labuschagne, M.T.; A. M. Deswardt and C. D. Vilvovn (2000). Genetic relationship South African wheat cultivar as measured by gliadin banding pattern. Plant breeding, 119-208.

Ohms, J. (1985). Determination of the maternal type in hybrid maize seed by isoelectric focusing of corn protein 2nd International Symposium "Cultivar Idintification and Evaluation" Abstr. 9. Braushweig, west Germany:

Rao, K. V; P. Supra Sanna; G.M. Reddy (1997). Differential expression of esterase and MDH isozymes during in vitro culture in maize (*zea mays, L*): Acta- physiologiae- plantarum. 19: 1,29-32.

Revin, E. V. and A.I. Rotar. (1981). Perixidase isozyme patterns during grain germination in high- lysine and normal forms of maize. P1. Breed. Abst. 52: 4725.

SPSS (1995). SPSS user 's guide. SPSS. Inc. USA.

Studier, F. W.(1973). Analysis of bacteriophage T1 early RNAs and proteins of slab gels. J. Mol. Biol., 79: 23-7248.

Tanksely, S.D. and C.M. Rick, (1980). Isoenzyme gene linkage map of the tomato: Applications in genetics and breeding. Theort. Appl. Genet., 57: 161-170.

Vierling, R.A. and H. T. Nguyen (1992). Use of RAPD marker to determine the genetic diversity of diploid wheat genotypes. Theor. Appl. Genet., 84: 835- 838.

Zhang, W.Q., and X.Z. Tang (1983). A study of isozymes in maize mutants induced by irradiation. Application of atomic Energy in Agric. 10:14-17.

تعدد المظاهر الوراثية لبروتينات الحبوب المختزنة والمتشابهات الإنزيمية في الذرة الشامية

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و هاني السيد عبد الحميد محمد دسوقي

قسم الوراثة – كلية الزراعة – جامعة الزقازيق

أمكن التمييز بين سلالات وهجن الجيل الأول في الذرة الشامية باستخدام التفريد الكهربي للبروتينات المخزونة والمشابهات الإنزيمية في الحبوب. وأعطى التفريد ألكهربي أثنى وعشرون حزمة من البروتينات الذائبة في الكحول موجودة في كل التراكيب الوراثية وجود اختلافات في الكثافة. أمكن تعين ثماني حزم مشتركة في كل التراكيب الوراثية وكان عدد الحزم البروتينية في طرز الهجرة الكهربية لأغلب هجن الجيل الأول أكثر عدداً منها في الآباء وكما أمكن تعين عشرة حزم مشتركة في التراكيب الوراثية لكل من إنزيمي : البروكسيديز والإستريز.

الـ **Dendrogram** يصف العلاقة بين الواحد والعشرون في التراكيب الوراثية للذرة الشامية التي أظهرت وجود مجموعتين رئيسيتين باستخدام نتائج البر وتينات والمشابهات الإنزيمية. وأمكن الاستفادة من البيانات المتحصل عليها في التفريق بين الأصناف.