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BIOCHEMICAL AND MOLECULAR DIVERSITY AND THEIR RELATIONSHIP TO LATE WILT DISEASE RESISTANCE IN YELLOW MAIZE INBRED LINES

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Maize (*Zea mays* L.) is considered the third in production among the major cereal crops after wheat and rice, therefore it has the highest genetic yield potential. It is one of highly affected crops with diseases. These diseases affect the quantity and quality of grains and reduce their production which may increase cultivation costs. Diseases affecting maize caused about 9% loss in yield (Khokhar *et al.*, 2014).

Late wilt of maize, caused by *Cephalosporium maydis*, is the most economically important fungal disease of maize in Egypt (El-Shafey and Claflin, 1999). This disease was discovered in Egypt for the first time in 1960 (Sabet *et al.*, 1961) and spread rapidly since its discovery and is now considered endemic throughout Egypt. The grown cultivars and the degree of soil infestation are the two main reasons affecting yield loss. In infested fields, up to 80% of the suscepti-

ble plants may become infected, and grain yield losses may reach 37% of wilted plants, and about 15% of the total yield in Egypt (Samra *et al*., 1971).

The most economically effective management and best control of late wilt disease is through development and using resistant germplasm and subsequently development of genetically resistant maize inbred lines (Zeller *et al*., 2000).

Traditional qualitative genetic approaches were used to study the nature of this disease and proved that resistance is under polygenic control; however, one study claimed resistance was controlled by a single dominant gene (García-Carneros *et al*., 2012). Meanwhile, El-Itriby *et al*. (1984) reported that resistance to late wilt disease was partially dominant with five loci controlling resistance and additive effect controlled by three major genes. Discover of specific genetic markers for late wilt resistance may strongly facilitate the induction of late wilt resistant hybrids (Drori *et al*., 2012).

The use of biochemical markers based on enzyme found to be a useful and inexpensive approache of developing genetic markers for assessment of genetic diversity of several plant species. They detect differences among enzymes that expressed by different alleles at one or more gene loci (isozymes) (Oppong-Konadu *et al*., 2005).

Esterase plays an important role in plant growth and development, cell wall expansion, stomatal movement and resistance against pathogen infection. Isoesterase intensity increasing was detected in several plants such as bean and barley leaves upon infection by different fungal pathogens (Rudolph and Stahmann, 1966). Varier and Cook (1992) used isoelectric focusing and polyacrylamide gel electrophoresis of seed esterases for pearl millet cultivar discrimination and identification.

The role of peroxidase activity in plant diseases resistance has been studied for several patho-systems in plants (Tornero *et al*., 2002; Carvalho *et al*., 2006). Peroxidase was determined as one of the first enzymes responding and providing fast defense against plant pathogens (Sulman *et al*., 2001). Due to infection with plant pathogens, an induction in peroxidase activity in several plant tissues and a greater increase was observed in resistant plants compared to the susceptible ones (Mydlarz and Harvell, 2006). Infiltration of leaves with a commercial preparation of peroxidase or even its direct activation was recorded to protect susceptible plants against plant pathogens (Converso and Fernandez, 1996). Such antifungal effect of peroxidase was suggested to be due to certain peroxidase isozymes (Caruso *et al*., 2001).

Molecular genetic markers are strongly used for the assessment of variability at the DNA level. Among them, RAPD marker (Random Amplified Polymorphic DNA) is the most accessible and has been used in wide range of plant crops to assess genetic diversity and evaluate

genetic relationship between genotypes (Rafalski and Tingey, 1993). Principal coordinate analysis (PCoA) was used for identifying the genetic similarity and quantitative variation in crops (Rahim *et al.*, 2008; Sesli and Yeğenoğlu, 2010). This analysis classifies the genotypes into various groups on the basis of geographic origin comparatively in a better way than the UPGMA clustering (Selvaraj *et al.*, 2010).

This study aimed to detect the relationship between genetic polymorphism and late wilt resistance in 18 yellow maize inbred lines and to identify the biochemical and molecular markers related to late wilt disease resistance using esterase, peroxidase and RAPD markers to enhance future breeding programs.

MATERIALS AND METHEDS

Plant material and experimental design

Evaluation of 18 yellow maize inbred lines (L-1 to L-18) for their susceptibility to the infection by *C. maydis* was carried out under artificial infestation in disease nurseries during three growing summer seasons; 2015, 2016 and 2017, at Sakha Agriculture Research Station. All inbred lines used in this study were obtained from Maize Breeding Program, ARC, Giza, Egypt (Table 1). Randomized complete block design (RCBD) with six replications was used in three years. Plot size was one row, 6 m. long and 80 cm. apart with 25 cm. between hills. One plant was left per hill after thinning at 21 days from planting. All agronomic field operations were practiced as usual with ordinary field maize cultivation.

Data for resistance to late wilt were collected as number of resistant plants per plot at 40 days after mid-silking or after 105 days from planting according to Sabet *et al*. (1961), then adjusted as percentages of the total number of plants per plot. The angular transformation of the data was performed according to Snedecor and Cochran (1967). The scale of resistance was suggested by El-Shafey *et al*. (1988), which reported that the infection from 0-5% was considered as highly resistance, from 5.1-10% as resistance, from 10.1-15% as moderately resistance and more than 15% as susceptible.

Assessment of genetic diversity

Isozymes and molecular analyses in the present study were conducted at the laboratories of Genetics Department, Faculty of Agriculture, Kafr El-Sheikh University, Egypt.

a. Isozyme analyses

For extraction of isozymes, 0.5 g fresh leaves from each sample was crushed in liquid nitrogen in an ice chilled mortar and pestle with 3.0 ml of ice cold extraction buffer {300 ml of 50 mM Tris/Hcl buffer (PH=7.8) containing 0.11 g EDTA-disodium salt and 22.5 g soluble polyvinylpyrrolidone (PVPP)}. The extract was centrifuged twice at 12,000 rpm for 15 min. at 4°C and the supernatant was separated. Polyacrylamide gel elec-

trophoresis (PAGE) according to Laemmli (1970) was used for qualitative analysis of enzymes. Estrase (EST) isoforms were detected on the gel using α-naphthyl acetate as substrate and subsequent color was developed with fast blue RR salts (Scandalios, 1969). While, peroxidase (PRX) isoforms were detected according to Scandalios (1964) in 0.25% benzidine dihydrochloride and 0.30% hydrogen peroxide.

Stained gels were placed in a light box to determine their isozyme banding patterns. The visual bands were recorded and the gels were photographed. The number of bands was recorded and their relative mobilities (Rf) were determined using GelAnalyzer 2010a software.

b. Molecular analysis

DNA extraction

Total genomic DNA was extracted from fresh leaves of 18 yellow maize inbred lines using Cetyl trimethyl ammonium bromide (CTAB)-based procedure as described by Murray and Thompson (1980) and the DNA was quantified on 1.5% agarose gel using standard DNA ladder.

RAPD primers and amplification conditions

Genomic DNA was used as a template for PCR (Polymerase Chain Reaction) amplification using 15 randomly selected primers (Operon Technology, USA). Fifteen 10-mers arbitrary RAPD primers (OPA-09, OPA-14, OPA-20, OPB-01, OPB-07, OPB-08, OPB-10, OPB-11, OPB-12, OPB-17, OPH-01, OPH-02, OPH-03, OPH-04 and OPH-05) were screened for studying genetic diversity among 18 maize inbred lines which differ in response to late wilt disease resistance. Amplification reactions were performed in a 20 µl volume containing 10 µl of master mix (2x PCR Master mix solution (i-Taq^{TM}) iNtRON Biotechnology, Korea), 1 µl of primer 10 pmol, 8 µl of double distilled water and 40 ng of template DNA. The reaction mixtures were overlaid with 20 µl of mineral oil per sample. The PCR amplification was performed on a thermal cycler (Perkin Elmer Cetus) programmed for a first denaturation step of 5 min at 94°C followed by 40 cycles of 1 min at 94°C, 1 min annealing at 30°C, 1.30 min extension at 72°C. The final stage was kept at extension temperature of 72°C for 7 min and then held at 4°C until the tubes were removed. Amplification products were analyzed by electrophoresis and detected on Benchtop UV-transilluminator and photographed using photoDoc-It^{TM} Imaging System. The molecular size of the amplified products was determined against O'GeneRuler DNA Ladder Mix, ready-to-use (Thermo Scientific).

Scoring and analysis of isozymes and RAPD data

Data matrices were created from photographs of gels and analyzed using GelAnalyzer 2010a program. These data assigning 1 to visible band and 0 to absent

band for each primer and entered in the form of a binary data matrix. From this matrix, cluster analysis and principal co-ordinate analysis (PCoA) were performed using Nei & Li coefficients (Nei and Li, 1979) by computational package MVSP 3.1. Data were processed by cluster analysis using the unweighted pair group average method (UPGMA) and plotted in the form of dendrogram using the same software.

RESULTS AND DISCUSSION

Analysis of variance and mean performance of resistance to late wilt disease

Analysis of variance for percentage of resistance to late wilt disease across three years is presented in Table (2). Mean square due to years (Y) showed highly significant differences. This result means that markedly changes in resistance were different from one year to another. Also, genotypes (G) and their interaction with year (G×Y) represented highly significant differences indicating that wide diversity was found among 18 inbred lines in relation to their susceptibility to this disease.

Mean performance for percentage of resistance to late wilt disease as an average of three years are shown in Table (3). Means ranged from 79.67% (L-16) to 97.50% (L-3) with an average of 88.59%. On the otherwise, the classification of inbred lines showed that five inbred lines (L-3, L-4, L-7, L-12 and L-18) showed highly resistance against this disease. Also, other five inbred lines (L-1, L-2, L-6, L-15 and L-17) were resistant. Meanwhile, four inbred lines (L-9, L-10, L-11 and L-14) exhibited moderately resistance. The rest four inbred lines (L-5, L-8, L-13 and L-16) were susceptible to this disease. These results agree with El-Mehalawy *et al*. (2004) who reported that success on a field scale has not been demonstrated consistently. On the other hand, new pathogen strains of *Harpophora maydis* have developed so that breeding for resistance for late wilt will remain a continuous process. The most effective control of late wilt disease is resistant genotypes (Zeller *et al*., 2000), and some cultural and chemical controls can reduce its impact on commercial production. Although, *H. maydis* lineages differ in their ability to colonize maize plants and in their relative aggressiveness in single culture inoculations (Zeller *et al*., 2002). The development of specific genetic markers for resistance to late wilt would greatly facilitate incorporation of resistance into different genotypes.

Assessment of genetic diversity as revealed by isozyme analysis

Isozymes are multiple molecular forms with similar or identical substrate specificity occurring within the same organism. The difference in the isozyme pattern can be used as a laboratory tool in the biochemical characterization of resistance and susceptibility of host plant.

a. Esterase (EST) isozyme analysis

Isozyme patterns of EST were determined and analyzed in the 18 maize

inbred lines under study as shown in Fig. (1) and Table (4). In respect of the EST zymograms, a view of sub-patterns was observed among the different tested genotypes. No monomorphic bands were detected in all of the 18 tested inbred lines. The 20 exhibited bands were polymorphic including five unique bands with relative mobility of 0.233, 0.481, 0.522, 0.752 and 0.789. They were absent from all genotypes and presented in three inbreed lines; one in the susceptible line (L-13) and two in the resistant genotype (L-6) and the other two in the moderate genotype (L-9). These differences among genotypes will help in breeding programs using different strategy.

Esterase isozyme has been used as genetic marker with resistance in wheat genotypes (Das *et al.*, 2002). They found that well-formed esterase isozyme bands were determined in healthy and inoculated seedlings of all wheat genotypes in response to infection with spot blotch. Resistant genotypes indicate high diversity in both healthy and inoculated seedlings. However, in disease causing fungus inoculated seedlings, two bands were observed differing distinctly in healthy seedlings. In moderately resistant genotypes, three extra bands appeared in infected seedlings. Prasad *et al.* (2003) studied isozymes variability among *Fusarium udum* resistant cultivars of pigeon pea (*Cajanus cajan* L. Millsp). The significant differences were observed in the leaf phosphatase and esterase banding profiles of all the cultivars. The presence of leaf esterase band at Em of 0.3 was well expressed in ICP 8863 (resistant) as compared to other cultivars.

b. Peroxidase (PRX) isozyme analysis

The results of electrophoretic banding patterns of peroxidase (PRX) isozyme analysis for the 18 maize inbred lines under study are shown in Fig. (2) and Table (5). All of the tested lines exhibited differences in both number and activity of bands. The detection of PRX patterns in the 18 tested genotypes was distributed from two to five bands and no monomorphic bands were detected.

Thirteen bands were polymorphic and presented in both resistant and susceptible lines with different activities and the relative mobility of the 13 bands ranged from 0.091 to 0.880, although some of these bands showed over expression in some tested lines. Two unique out of 13 bands were seen in the moderate resistant genotype (L-10) and the susceptible genotype (L-13) only. These variations in isozyme patterns which were detected in peroxidase zymograms reflect the differences in genetic background for the tested lines.

Peroxidase isozyme has been widely used as genetic marker, since it presents different isoforms in most tissues (Lara *et al.*, 2003). Peroxidase isozymes also play an important role in the biosynthesis of cell wall components, as well as cellular differentiation (Christensen *et al*., 1998) and their relationship with resistance to adverse biotic and abiotic factors (Dalisay and Kuc, 1995). Increased

peroxidase activity and induction of new isoenzyme have been observed in cotton bolls inoculated with *R. solani* (Mellon and Lee, 1985). Prasad *et al.* (2003) analyzed the isozymes variability among *Fusarium udum* resistant cultivars of pigeon pea (*Cajanus cajan* L.) and reported that one specific leaf peroxidase band and two root peroxidase bands were induced in resistant genotype (ICP 8863). Similar results showing increase in intensity of peroxydase isoform were reported by Chandra and Rajan (2000) in tomato upon *Pseudomonas Solanacearum* infection.

Assessment of genetic diversity using RAPD assay

The present study demonstrates potential use of RAPD-PCR based molecular markers to differentiate maize genotypes differing in their reaction to late wilt and results are presented in Table (6).

Difference in banding patterns among the resistant and susceptible genotypes was marked. A total of 15 RAPD primers were evaluated for their ability to prime PCR amplification of maize genomic DNA of 18 inbred lines varied in their response to late wilt disease. A total of 262 RAPD loci were amplified from the different maize genotypes. The PCR products were in the molecular size range of 120-4167 bp and the number of amplicons per primer varied from 10 (OPB-01) to 22 (OPA-09 and OPH-04) with an average of 17.47 loci per RAPD primer (Fig. 3 and Table 6). Out of the 262 loci scored, 218 loci (83.21%) were found to be polymorphic and 44 loci (16.79%) were found to be monomorphic in nature with an average of 14.53 polymorphic loci per primer (Table 6). The polymorphism (%) ranged between 33.33 (for OPH-03) and 100% (for OPA-14). Moreover, all primers produced positive unique DNA bands, except OPB-11. The average polymorphic information content (PIC) of the 15 RAPD primers was 0.25, ranging from 0.07 to 0.36 (Table 6). The lowest and highest PIC values were recorded for primers OPH-03 and OPB-11, respectively.

Kotresh *et al.* (2006) reported the presence of two amplicons at 704 bp and 500 bp (OPM03704 and OPAC11500) with susceptibility, while, Prasanthi *et al.* (2009) identified specific amplification for resistance to late wilt at 920 bp with OPG08 primer (OPG08920). Dutta *et al.* (2011) reported that RAPD could differentiate the genotypes but clear relationship between resistant and susceptible groups was absent.

Cluster analysis and principal coordinate analysis (PCoA)

Cluster analysis and principal coordinate analysis (PCoA) for the 18 maize genotypes were performed based on the relative genetic distances from esterase, peroxidase and RAPD data. The PCoA determines the consistency of the differentiation among the genotypes defined by the cluster analysis (Adhikari *et al*., 2015).

a. Based on esterase analysis

Cluster analysis based on esterase data indicated that the genetic distances among all the maize genotypes were very high indicating that degree of similarity was low among these genotypes. The UPGMA analysis divided the tested genotypes into four groups at similarity percentage of 9.7%. Group I contain six inbred lines that were differed in reaction to late wilt (Fig. 4), included three Egyptian genotypes, two Serbia and one from Thailand. The four genotypes in Group II had moderate, resistant and highly resistant ratings for late wilt and these genotypes were originated from USA×Egypt, Thailand and CIMMYT. The five genotypes in Group III had susceptible and highly resistant ratings to late wilt (one from Jugoslavija, two from USA and two hybrid USA×Egypt). Three genotypes (USA, Egypt and one Egyptian hybrid) in Group IV had resistant and moderate ratings for late wilt. The results also showed that both of (L-1 and L-2) and (L-3 and L-4) showed 100% genetic similarity.

Principal coordinate analysis (PCoA) based on esterase data has managed to divide these genotypes into three groups. The first eigenvector (PCoA axis1) accounted for 23.38% of the data variation among groups (Fig. 5). The second eigenvector (PCoA axis2) explained 18.73% of the variation. This value indicated that the PCoA succeeded in assessment of genetic diversity and description of heterogeneity within the studied genotypes. These results agree with those reported by Sonja *et al*. (2008) and Abd El-Aziz *et al*. (2016).

b. Based on peroxidase analysis

The hierarchical cluster analysis of peroxidase isoforms for the 18 maize inbred lines under study produced two main clusters containing four groups on similarity percentage of 10.6% between clusters (Fig. 6). Group I included three different genotypes that were from Egypt, Serbia and CYMMYT. Group II included six inbred lines, three of them were Egyptian genotypes (L-10, L-13 and L-14), that were relatively moderate and susceptible to late wilt disease. The other three genotypes in Group II had high resistant ratings for late wilt in L-12 (from CIMMYT) and L-18 (from Thailand) and susceptible rating for L-16 from Serbia. The six genotypes in Group III had different ratings to late wilt disease; two high resistant genotypes (Jugoslavija and USA) and two susceptible genotypes (Hybrid USA×Egypt), one resistant hybrid (USA×Egypt) and one moderate genotype from Thailand. Late wilt resistance ratings were relatively high for L-3 (USA) and resistant for L-1 (USA) and L-2 Egyptian hybrid that formed Group IV.

Principal coordinate analysis (PCoA) based on peroxidase data for 18 inbred lines is shown in Fig. (7). The first eigenvector (PCoA axis 1) accounted for 40.88% of the data variation among groups. The second eigenvector (PCoA axis 2) explained 18.29% of the variation. This value indicated that the PCoA succeeded in assessment of genetic diversity

and description of heterogeneity within the studied genotypes. Principal coordinate analysis (PCoA) separated the 18 inbreed lines into three groups. The first group; comprised of nine genotypes, differ in their response to late wilt disease (three highly resistant, three resistant, one moderate and two susceptible). It contained all genotypes exotic from USA (three) and all hybrids including the three hybrids with parents from USA (three). The second group contained only L-10 from Egypt. The rest genotypes (eight) were clustered in the third group (Fig. 7) which contained all genotypes exotic from Serbia, CIMMYT and three Egyptian genotypes and one from Thailand. These results agree with those reported by Sonja *et al*. (2008) and Abd El-Aziz *et al.* (2016).

c. Based on RAPD analysis

The results presented in Fig. (8 and 9) showed the UPGMA clustering dendrogram and principal coordinate analysis (PCoA) based on binary data. These results indicated that the maize inbred lines divided into three groups at similarity percentage of 66% with different degrees of similarity. The first group (I) is comprised by the moderate inbred line L-11 only. The second group (II) is comprised of ten inbred lines having different rating for late wilt resistance from susceptible to highly resistant and including all Egyptian inbred lines (four), all exotic genotypes from Serbia (two), all exotic genotypes from Thailand (two), one genotype from CIMMYT and one hybrid; USA×Egypt. Seven inbred lines were presented in group III (three highly resistant, three resistant and one susceptible) including three genotypes from USA, two hybrids USA×Egypt, one Egyptian hybrid and one genotype exotic from Jugoslavija.

As well as, the results of principal coordinate (PCo) analysis gave clear indications for genetic diversity and divided the 18 inbred lines into two groups. The results showed that the first and second eigenvectors were 49.63 and 7.76% for PCoA based on RAPD analysis. The L-11 inbred line was separated in the first group alone. The second group contained all rest genotypes (17 inbred lines).

The selected primers used in this study succeeded in the production of various amplicons effective enough to reveal usable level of DNA polymorphism. But they were not effective enough to distinguish some inbred lines by unique markers. However, the obtained high level of polymorphism depends upon the degree of divergence between all studied inbred lines. This finding was in agreement with those stated by Aboulila (2016) and Abd El-Aziz *et al.* (2016). The results presented in Fig. (8) indicated that the studied inbred lines were divided into two groups with different degrees of genetic similarity of maize genotypes and this is necessary for identifying diverse inbred lines combinations that result in segregating progeny with high genetic variability for selection. In this respect Lanza *et al.* (1997) reported that RAPD technique can

be used as a good alternative tool to determine genetic diversity in maize. From the obtained data of infection response and UPGMA clustering dendrogram based on RAPD analysis, it is clear that the results are not in the same line for all genotypes. However, evaluating studied inbred lines in multi locations could make the method even more effective and give reliable results; because the discrimination ability increased with increasing heritability for traits. Also, these results indicated that the studied inbred lines can be divided into the same groups in UPGMA cluster analysis based on RAPD analysis. Based on that, the cluster analysis was supported by principal coordinate analysis (PCoA). These results were in agreement with those obtained by Aboulila (2016) and Abd El-Aziz *et al.* (2016).

Genotype-specific markers using RAPD data

Inbred lines specific markers generated from RAPD-PCR analysis are presented in Table (7). Forty five out of 2191 amplified bands were found to be useful as unique markers (38 positive and seven negative unique markers). All primers generated positive unique markers, except OPB-11. The highest number of unique markers was generated by primer OPA-14 (seven positive and one negative unique marker), while the lowest number (one unique marker) was generated by primers OPB-01, OPH-01 and OPH-03 (positive unique marker), in addition OPB-11 (negative unique marker). On the other hand, the largest number of unique markers was scored for L-11 inbred line (14 unique markers). L-15 inbred line was identified by the highest number of amplified bands (152) followed by L-11 (143). On the other hand, L-1 and L-13 did not show any unique markers.

In this study, we have identified genotype-specific molecular markers, which might contain some important gene sequences that could be used for the development of molecular studies. The molecular nature of the polymorphisms can be known only if the fragments extracted from the gel are sequenced. With the increasing ease and speed of DNA sequencing alongside decreasing costs, DNA profiling will facilitate rapid and large-scale biodiversity surveys, both for inventory purposes and ecological studies. This could be performed without presorting of samples or the necessity for taxonomists to devote their time to highly repetitive identification rather than additional scientific research. It would allow the identification of different life stages, e.g., seeds or seedlings and fragments of plant material that do not bear the requisite morphological characters for identification and without carrying out any extensive biochemical and cytological studies. These results were in the same line of Adhikari *et al*. (2015).

In conclusion, it was of great interest to mention that (L-1 and L-2) and (L-3 and L-4) showed the same results for late wilt infection response. These genotypes showed also the same results in the expression of esterase isozymes. According

to molecular studies, the same genotypes were always isolated together in cluster analysis and also in a specific position in principal coordinate analysis. The results of this study suggest that the polymorphism observed among the isozymes (esterase and peroxidase) is very informative and useful for disease resistance studied. RAPD technique was efficient in detecting polymorphism and determining genetic diversity among the 18 studied inbred lines. Nonetheless, the genetic similarity did not correlate significantly with the infection response studied trait. But the differences among genotypes were very important in breeding programs. However, screening of more primers and also study with the help of gene specific markers may provide useful information for resistances and susceptibility reactions with a particular genotype.

SUMMARY

In an attempt to assess the genetic diversity among 18 maize inbred lines used in breeding programs and to identify specific genetic markers for late wilt disease resistance, esterase, peroxidase and RAPD markers were used. Fifteen RAPD primers were successful for evaluation of 18 inbred lines of maize. These biochemical and molecular techniques were efficient in detecting genetic polymorphism with an average of 100, 100 and 83.21% for esterase, peroxidase and RAPD, respectively. For cluster analysis, the 18 inbred lines were divided into four groups based on esterase and peroxidase isozyme and three groups based on RAPD analysis. Also, the principal coordinate analysis separated the 18 inbred lines for three groups (esterase and peroxidase analyses) and two groups (RAPD analysis) and the separation was according to the imported location more than the late wilt disease resistance. On the other hand, 45 out of 2191 amplified bands were found to be useful as unique markers. All primers produced genotype specific markers, except OPB-11. Also, L-11 maize inbred line scored the highest number of genotype specific markers (14 unique markers) and these markers may help breeders for selection of late wilt resistant maize genotypes.

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Table (1): Names, sources and origin of 18 yellow maize inbreed lines used in this study.

Name	Source	Origin	Name	Source	Origin
L-1	Moll17×SC3084	USA	L-10	Pop. Gm7421	Egypt
L-2	SCGm1004×Sk6241	Egypt×Egypt	L-11	CIMMYTE2010	CIMMYT
L-3	Exotic Pop. Drought	USA	L-12	CIMMYTE2010	CIMMYT
L-4	Exotic-2003	Jugoslavija	L-13	Pop. $SK8S_1C_1$	Egypt
L-5	SCGm1021×Sd318	USA×Egypt	L-14	Pop. SK11R	Egypt
L-6	SCB73×Sd62	USA×Egypt	L-15	SCZP735	Serbia
L-7	Cargill922	USA	L-16	SCZP747	Serbia
L-8	Moll17×Sd7	USA×Egypt	L-17	Pop. SK13 Color	Egypt
L-9	DC201	Thailand	L-18	DMY207	Thailand

Table (2): Analysis of variance for percentage of resistance to late wilt disease across three years.

SOV	D.f.	MS
Years (Y)	2	3097.289**
Rep/Y	15	169.060
Genotypes (G)	17	709393**
G×Y	34	283.102**
Error	255	138.457

*,** significance differences at 5% and 1% levels of probability, respectively.

Table (3): Mean performances for percentage of resistance to late wilt disease as an average of three years.

Inbred lines	Resistance to late wilt %	Degree of resistance	Inbred lines	Resistance to late wilt %	Degree of resistance
L-1	92.06	R	L-10	86.56	M
L-2	94.22	R	L-11	89.78	M
L-3	97.50	HR	L-12	97.22	HR
L-4	96.00	HR	L-13	83.22	S
L-5	83.78	S	L-14	85.72	M
L-6	94.00	R	L-15	91.56	R
L-7	95.28	HR	L-16	79.67	S
L-8	84.67	S	L-17	90.78	R
L-9	87.28	M	L-18	96.83	HR
L.S.D. $_{0.05}$	7.68				
L.S.D. $_{0.01}$	9.96				

Table (4): Esterase (EST) isozyme banding pattern, relative mobility, band frequency and polymorphism of 18 maize inbred lines.

Isoform (locus)	RF	L-1	L-2	L-3	L-4	L-5	L-6	L-7	L-8	L-9	L-10	L-11	L-12	L-13	L-14	L-15	L-16	L-17	L-18	Frequency	Polymorphism
EST-1	0.116	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	0.167	Polymorphic
EST-2	0.169	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	+	+	0.278	Polymorphic
EST-3	0.194	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	0.167	Polymorphic
EST-4	0.233	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	0.056	Unique
EST-5	0.376	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	0.111	Polymorphic
EST-6	0.388	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	0.333	Polymorphic
EST-7	0.416	-	-	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	0.278	Polymorphic
EST-8	0.438	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	0.167	Polymorphic
EST-9	0.481	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	0.056	Unique
EST-10	0.522	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	0.056	Unique
EST-11	0.649	-	-	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	0.167	Polymorphic
EST-12	0.670	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	0.222	Polymorphic
EST-13	0.696	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	0.111	Polymorphic
EST-14	0.722	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	0.167	Polymorphic
EST-15	0.752	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	0.056	Unique
EST-16	0.789	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	0.056	Unique
EST-17	0.814	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-	+	-	-	0.222	Polymorphic
EST-18	0.858	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	0.222	Polymorphic
EST-19	0.899	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+	-	-	-	0.500	Polymorphic
EST-20	0.946	-	-	+	+	-	-	-	+	-	-	+	-	-	-	-	-	-	-	0.222	Polymorphic
Total		4	4	4	4	3	5	2	4	5	2	4	4	4	4	3	4	2	3		100 %

(+) present (-) absent

Table (5): Peroxidase (PRX) isozyme banding pattern, relative mobility, band frequency and polymorphism of 18 maize inbred lines.

Isoform (Locus)	RF	L-1	L-2	L-3	L-4	L-5	L-6	L-7	L-8	L-9	L-10	L-11	L-12	L-13	L-14	L-15	L-16	L-17	L-18	Frequency	Polymorphism
PRX-1	0.091	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	0.278	Polymorphic
PRX-2	0.106	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	0.167	Polymorphic
PRX-3	0.132	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	0.556	Polymorphic
PRX-4	0.152	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	0.111	Polymorphic
PRX-5	0.272	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	0.111	Polymorphic
PRX-6	0.284	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	+	0.278	Polymorphic
PRX-7	0.308	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	0.500	Polymorphic
PRX-8	0.328	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	0.111	Polymorphic
PRX-9	0.338	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	0.056	Unique
PRX-10	0.389	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	0.111	Polymorphic
PRX-11	0.509	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	0.056	Unique
PRX-12	0.653	+	+	+	-	-	-	-	-	-	+	+	+	+	-	+	+	-	+	0.556	Polymorphic
PRX-13	0.880	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	-	-	0.222	Polymorphic
Total		3	4	3	2	2	2	2	2	3	4	5	3	5	3	5	3	2	3		100 %

(+) present (-) absent

Table (6): RAPD primer sequences and distribution of amplified fragments in 18 inbreed lines of maize genotypes.

No.	Primer name	Primer sequence 5′→ 3′	Molecular size range (bp)	Mono-morphic bands	Polymorphic bands			Total num-ber of ampli-fied bands	Percentage of Poly-morphic loci	PIC
					without unique bands	Positive unique bands	Total			
1	OPA-09	GGGTAACGCC	169-1735	1	19	2	21	22	95.46	0.32
2	OPA-14	TCTGTGCTGG	157-4167	0	11	7	18	18	100	0.24
3	OPA-20	GTTGCGATCC	169-1333	1	16	4	20	21	95.24	0.29
4	OPB-01	GTTTCGCTCC	164-822	1	8	1	9	10	90.00	0.25
5	OPB-07	GGTGACGCAG	133-1679	3	15	2	17	20	85.00	0.25
6	OPB-08	GTCCACACGG	173-2848	3	8	4	12	15	80.00	0.25
7	OPB-10	CTGCTGGGAC	162-2140	4	13	1	14	18	77.78	0.23
8	OPB-11	GTAGACCCGT	144-2248	1	18	0	18	19	94.74	0.36
9	OPB-12	CCTTGACGCA	168-2060	4	8	5	13	17	76.47	0.20
10	OPB-17	AGGGAACGAG	185-3260	2	13	2	15	17	88.24	0.30
11	OPH-01	GGTCGGAGAA	157-1411	4	11	1	12	16	75.00	0.24
12	OPH-02	TCGGACGTGA	138-1907	2	11	4	15	17	88.24	0.26
13	OPH-03	AGACGTCCAC	141-2751	10	4	1	5	15	33.33	0.07
14	OPH-04	GGAAGTCGCC	120-2720	4	16	2	18	22	81.82	0.26
15	OPH-05	AGTCGTCCCC	227-1581	4	9	2	11	15	73.33	0.22
Total				44	180	38	218	262	83.21	3.74
Mean				2.93	12	2.53	14.53	17.47		0.25

Table (7): Total number of amplified fragments and specific markers for the 18 inbred lines of maize based on RAPD analysis.

Genotypes	Genotype specific markers			Total amplified bands
	Positive	Negetive	Total	
L-1	-	-	-	98
L-2	1333 bp (OPA-20)	-	1	127
L-3	237 bp (OPA-09) 1907 bp (OPH-02)	-	2	119
L-4	216 bp (OPH-04)	-	1	114
L-5	1447 bp (OPB-10) 2751 bp (OPH-03)	157 bp (OPA-14) 907 bp (OPB-12)	4	115
L-6	589 bp & 373 bp (OPA-20)	342 bp (OPA-20)	3	97
L-7	266 bp (OPH-02)	-	1	107
L-8	138 bp (OPH-02)	-	1	113
L-9	-	689 bp (OPH-04)	1	125
L-10	157 bp (OPH-01)	-	1	130
L-11	1735 bp (OPA-09) 4167 bp & 2221 bp & 799 bp & 470 bp (OPA-14) 2848 bp & 2298 bp & 1455 bp & 1214 bp (OPB-08) 2060 bp & 168 bp (OPB-12) 3260 bp (OPB-17) 501 bp (OPH-05)	493 bp (OPB-10)	14	143
L-12	285 bp (OPB-01)	-	1	129
L-13	-	-	-	121
L-14	293 bp (OPH-02) 120 bp (OPH-04)	-	2	134
L-15	2505 bp & 1841 bp & 1142 bp (OPA-14) 1436 bp (OPB-12)	-	4	152
L-16	466 bp (OPB-07) 432 bp (OPH-05)	544 bp (OPB-11) 385 bp (OPH-02)	4	113
L-17	156 bp (OPB-07) 2304 bp (OPB-17)	-	2	127
L-18	264 bp (OPA-20) 531 bp & 469 bp (OPB-12)	-	3	127
Total	38	7	45	2191

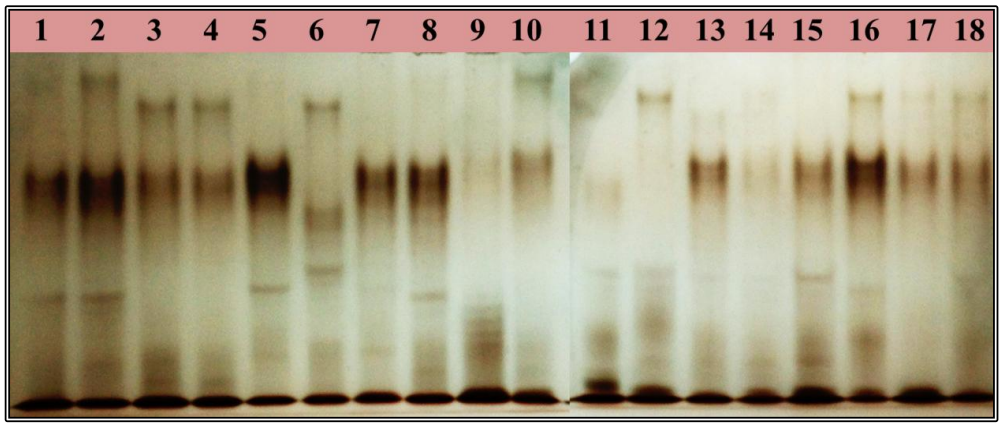

Fig. (1): Polyacrylamide gel zymogram of estrase isozyme banding patterns in the 18 maize inbred lines (L-1 to L-18) detected with α-naphthyl acetate substrate.

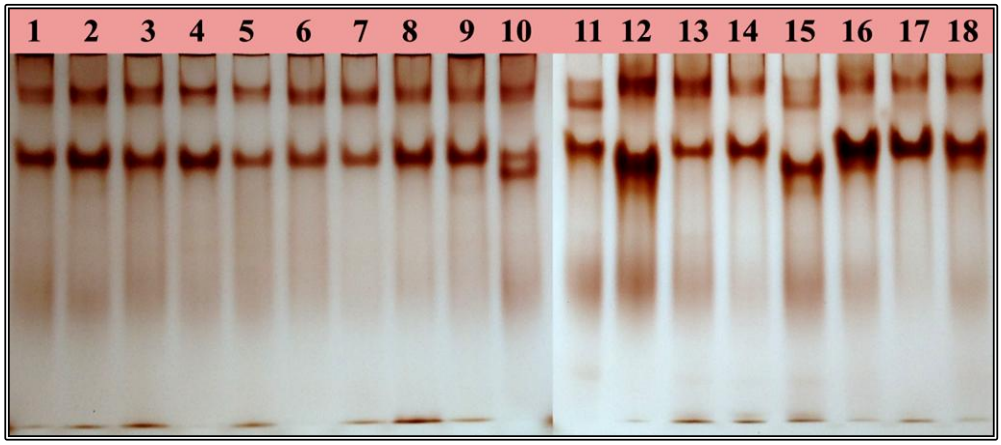

Fig. (2): Polyacrylamide gel zymogram of peroxidase isozyme banding patterns in the 18 maize inbred lines from L-1 to L-18**.**

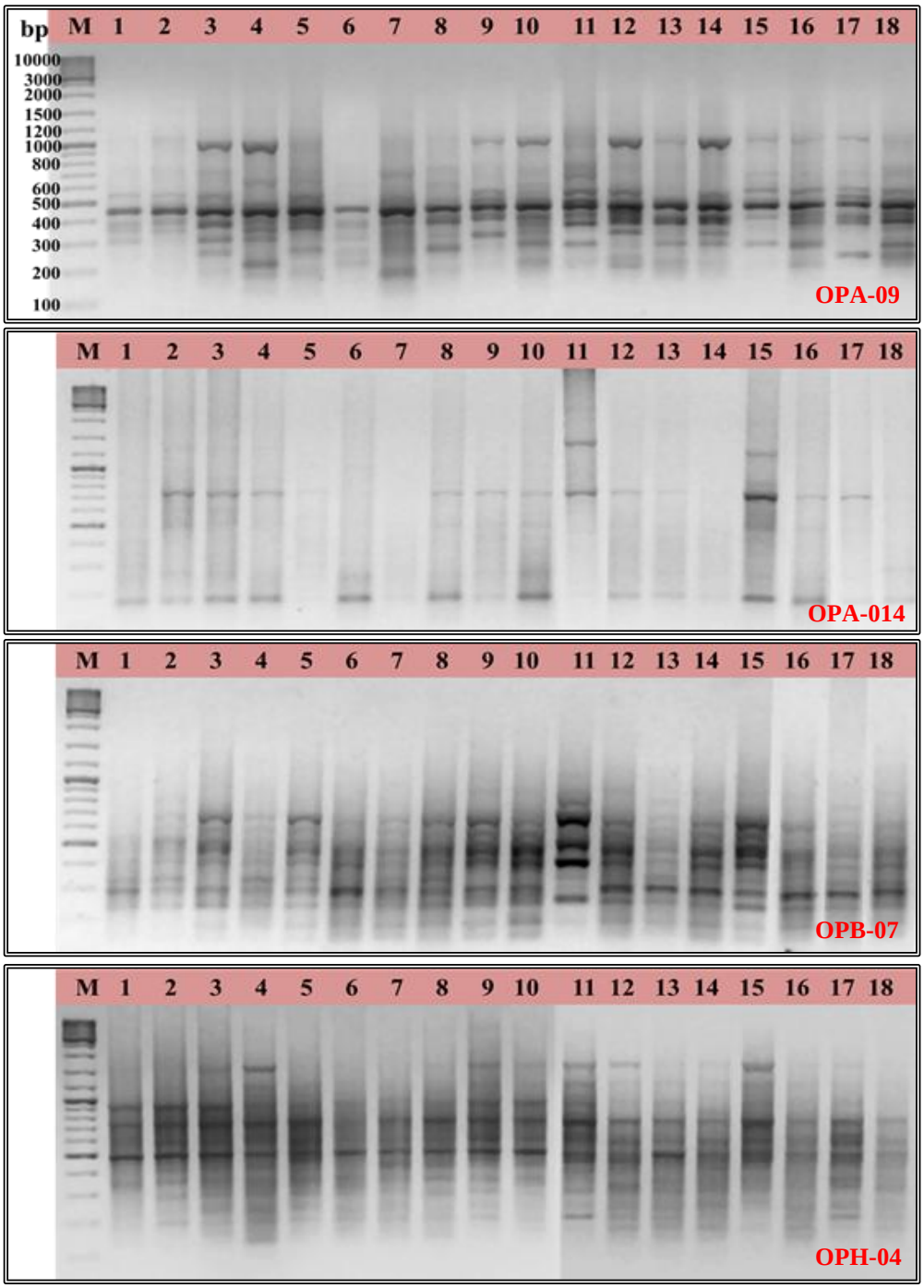

Fig. (3): RAPD fingerprints of the 18 maize inbreed lines with four RAPD primers (OPA-09, OPA-14, OPB-07 and OPH-04. M: O'GeneRuler DNA Ladder Mix and 1 to 18: maize inbred lines from L-1 to L-18.

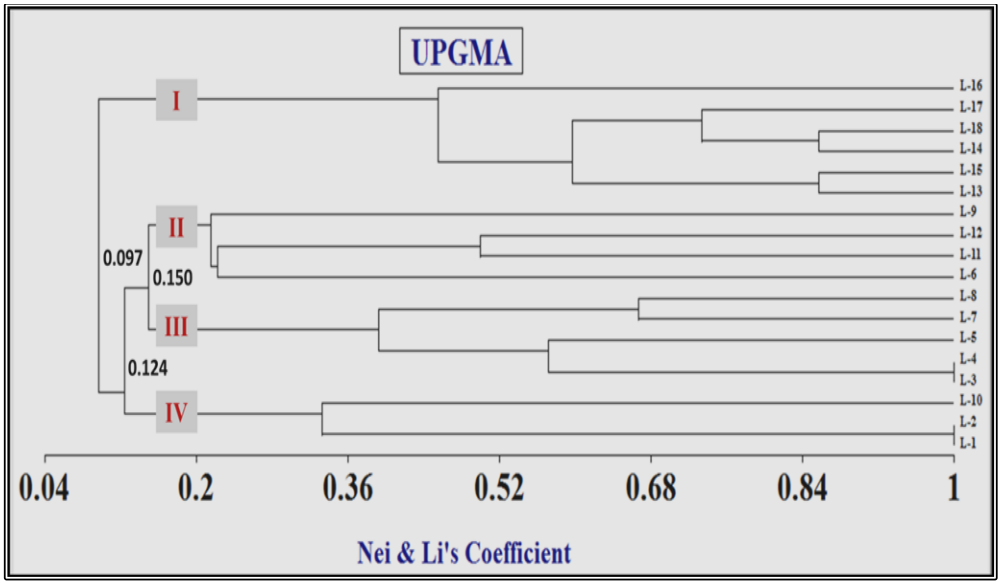

Fig. (4): Hierarchical cluster analysis of 18 maize inbred lines based on variation of esterase patterns.

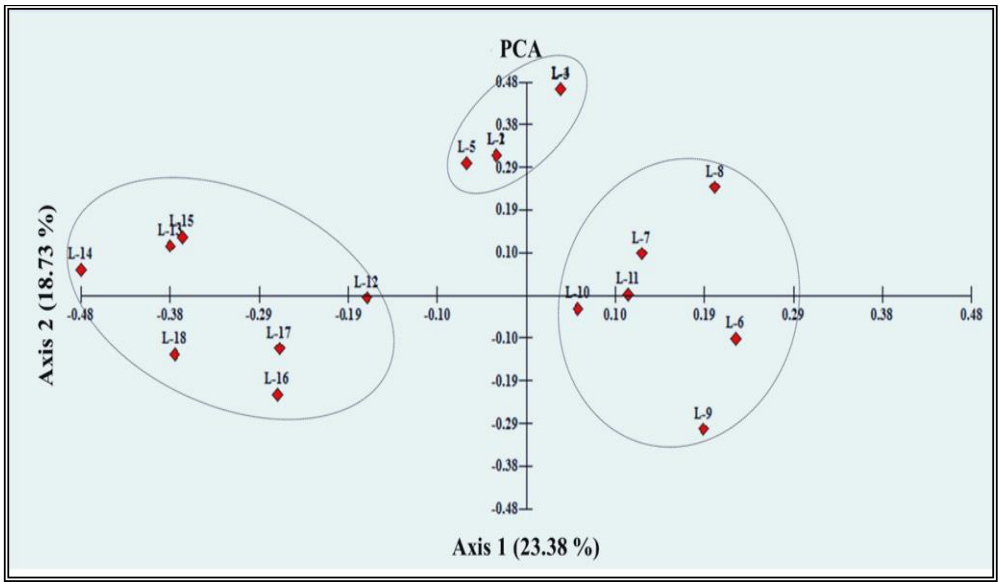

Fig. (5): Estrase marker-based principal coordinate analysis (PCoA) of 18 maize inbred lines.

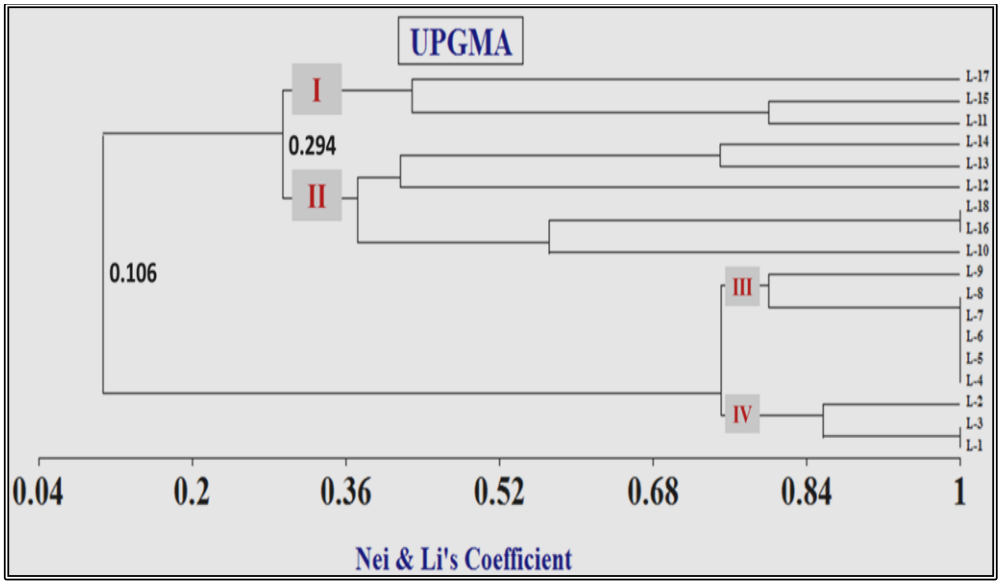

Fig. (6): Hierarchical cluster analysis of 18 maize inbred lines based on variation of peroxidase patterns.

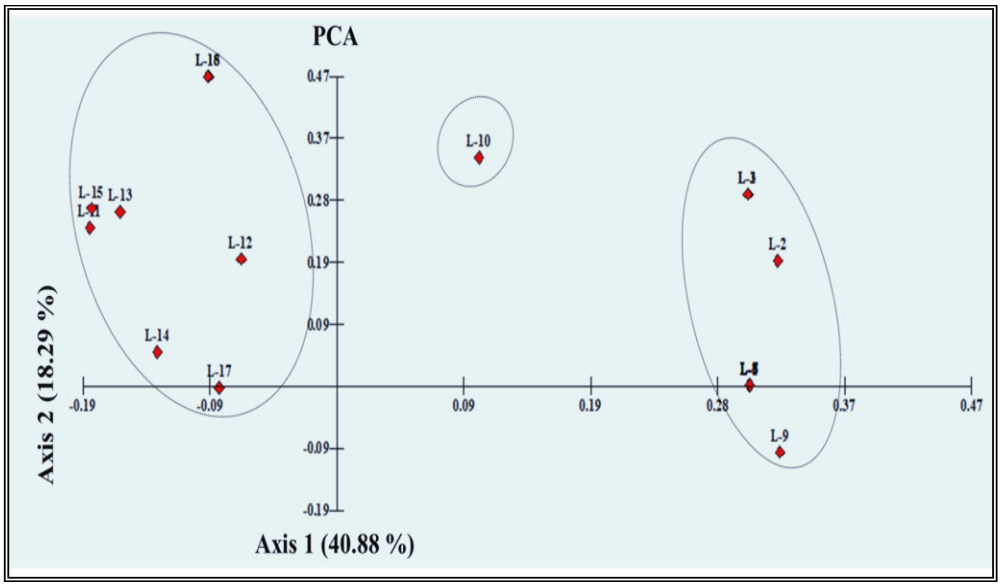

Fig. (7): Peroxidase marker-based principal coordinate analysis (PCoA) of 18 maize inbred lines.

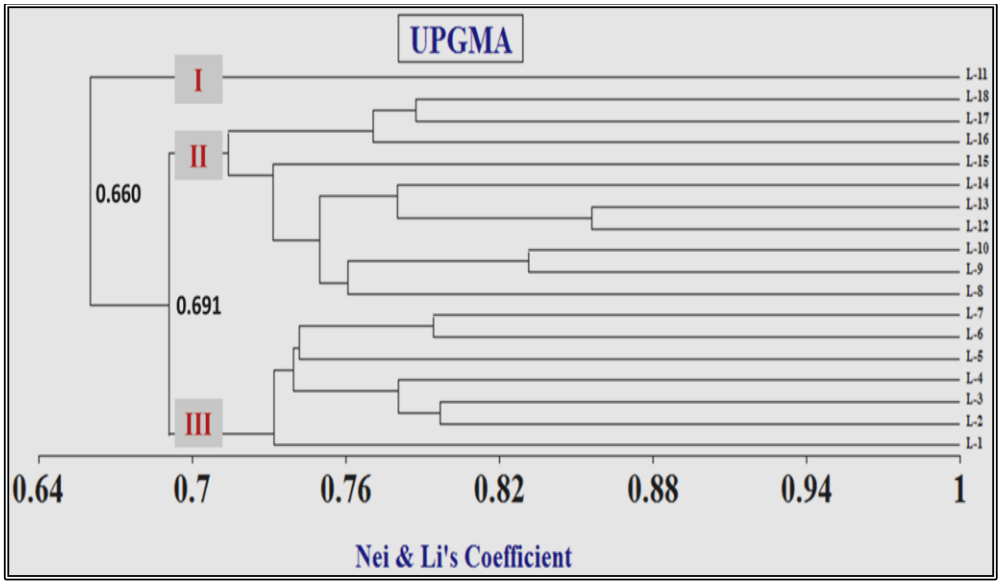

Fig. (8): Hierarchical cluster analysis of 18 maize inbred lines based on variations of RAPD patterns.

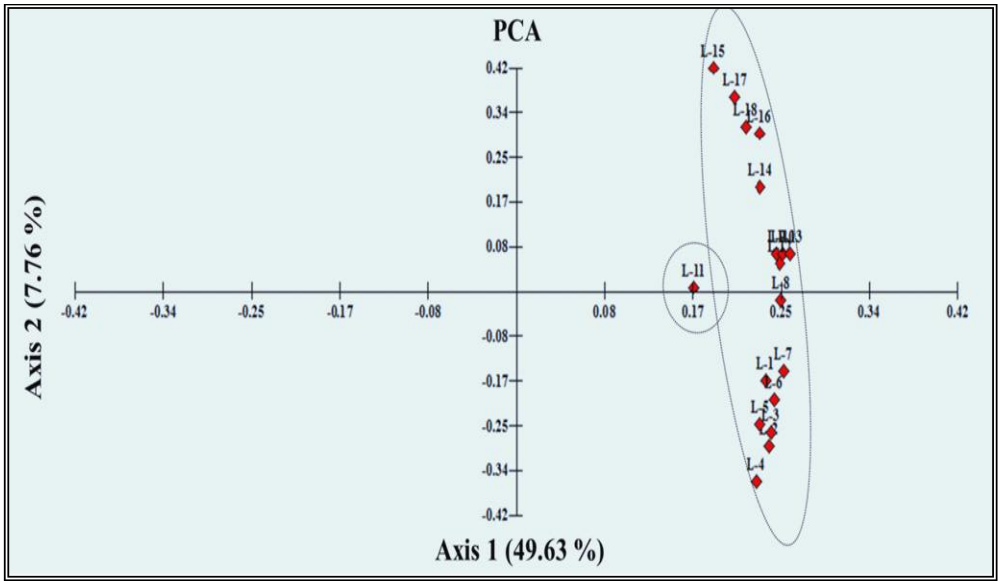

Fig. (9): RAPD marker-based principal coordinate analysis (PCoA) of 18 maize inbred lines.