MOLECULAR VARIATIONS OF MAIZE CMS POPULATIONS AND SUBPOPULATIONS

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ABSTRACT

RAPD DNA markers were used to evaluate trends in genetic diversity among 20 of 24 cms line populations derived from early and advanced cycles of selection. All of the 10 RAPD primers used for initial screening were found to be polymorphic. A total of 139 DNA fragments were generated by 10 random decamer primers, with an average of 13.9 easily scorable fragments per primer. The number of amplified fragments produced per primer ranged from 6 for OPC-02 primer to 21 for OPC-07 and OPE-07 primers, with molecular size ranged from 264bp to 2717bp. The total number of polymorphic fragments and the percentage of polymorphism were 109 and 78%, respectively. Maximum level of polymorphisms were (94%) and (93%) observed for the primers OPD-05 and OPC-08, respectively. Primers OPC-02 and OPD-08 showed the lowest percentage of polymorphism, which were about (50%) and (53%), respectively. Based on the bivariate (1-0) data and genetic similarity with the use of UPGMA cluster method, the dendrogram separated the studied populations into five major clusters I, II, III, IV and V. Cluster analysis which compared between original and subpopulations places in the dendrogram, showed that selection plays an important role in developing new populations via selfing. R6O and its progeny R6S showed a diverged genetic background against other populations. Genetic similarities, computed by Nei and Li's similarity coefficient revealed that the highest estimate (0.97) was observed between the original and subpopulation of R6. Meanwhile the lowest genetic similarity (0.76) was detected between the original and subpopulation of R2. The highest genetic similarity among the different populations was 0.89 observed between R3O and both R4 populations, whereas genetic distance widened slightly in R6 population after three cycles of selection as R6s possessed the lowest GS value (0.57) against A5O. Relationship between genetic diversity and hybrid vigor was fair enough. Results indicated that DNA molecular markers were highly efficient in detecting the purity and genetic relationship among maize cms populations.

Key words: Maize, CMS, RAPD Markers, Genetic Similarity

التغايرات الجزيئية لمجتمعات أصلية ومشتقة لسلالات عقيمة من الذرة الصفراء

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الخلاصة

أُستعملت معلمات RAPD لتقييم التغاير الوراثي بين عشرين من أصل أربعة وعشرين من مجتمعات العقم الذكري السايتوبلازمي في الذرة الصفراء الناتجة من دورات مبكرة ومتأخرة من الانتخاب. أنتجت جميع البادئات المستعملة في الغربلة الابتدائية حزماً متباينة. إذ بلغ مجموع الحزم الناتجة من استعمال البادئات العشر 139 حزمة، وبمعدل 13.9 حزمة للبادئ الواحد. تراوح عدد القطع المكوثرة بين 6 في البادئ OPC-02 و21 في البادئين OPC-07 و OPE-07، وبوزن جزيئي تراوح بين 264bp و2717bp. بلغ العدد الكلي والنسبة المئوية للقطع المتباينة 109 و78%، وبنفس التتابع. بلغت أعلى نسبة مئوية للقطع المتباينة 94% و93% للبادئين OPD-05 وOPC-08 وبنفس التتابع. اظهر البادئان OPC-02 و OPD-08 أوطأ نسبة مئوية للقطع المتباينة بلغت 50% و53% وبنفس التتابع. إعتماداً على البيانات الثنائية (0-1) والتشابه الوراثي بإستعمال طريقة UPGMA في إنشاء مخطط الصلة الوراثية، تم فصل المجتمعات ضمن خمس مجاميع: I وII وIII وIV وV. اظهر تحليل المجاميع الذي تمت فيه المقارنة بين أماكن المجتمعات الأصلية والمشتقة ضمن مخطط الصلة، أن الانتخاب بالتلقيح الذاتي قد لعب دوراً مهماً في إنتاج مجتمعات جديدة مغايرة. أظهر مجتمع R6O الأصلي وR6S الذي إنحدر منه تباعداً وراثيا مقارنة ببقية المجتمعات. بلغ التشابه الوراثي الذي تم تقديره وفق معامل Nei وLi أعلى قيمة (0.97) بين مجتمعي R6، الأصلي والمشتق، بينما بلغت أوطأ قيمه (0.76) بين مجتمعي R2 الأصلي والمشتق. بلغت أعلى قيمة للتشابه الوراثي بين المجتمعات المختلفة (0.89) بين المجتمعين R3O وR4O، بينما إزداد التباعد الوراثي قليلا في R6 بعد ثلاث دورات إنتخابية، إذ حاز R6S على أوطأ قيمة للتشابه الوراثي (0.57) مقابل مجتمع A5O. أشارت النتائج إلى إرتباط مقبول بين البعد الوراثي وقوة الهجين، وإن معلمات RADP ذات كفاءة عالية في تشخيص النقاوة والصلة الوراثية بين مجتمعات الذرة الصفراء العقيمة.

INTRODUCTION

The morphological and molecular characterization of maize genetic material would be critical for crop improvement, including genetic dissimilarity which is very important to corroborate genealogical relationships and to predict the most heterozygotic hybrid combinations, furthermore identification of heterotic groups in an indigenous germplasm (17). There are, however, serious challenges in identifying and describing the genes that control diverse phenotypes and adaptation of maize at these loci (14). Genetic knowledge of germplasm diversity among local maize populations has a significant impact on the improvement of these plants not only as a valuable source of useful traits, but also as a bank of highly adapted genotypes (1). The use of molecular markers is an invaluable tool in the arsenal of traditional plant breeding techniques, as they are useful in clarifying the number, chromosomal locations and genetic contribution of genes controlling complex (16). Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the identification of genetic polymorphism. In the last decade, the random amplified polymorphic DNA (RAPD) technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers. RAPD markers are amplification products of anonymous DNA sequences using single, short and arbitrary oligonucleotide primers, and thus do not require prior knowledge of a DNA sequence (2). The technique of RAPD gained importance due to its simplicity, efficiency, relative ease to perform and not requirement of sequence information (12). DNA sequences are independent of environmental conditions, furthermore, identification can be determined at any stage of plant growth (5), and among even very similar genotypes, including clonal variants can be discriminated by using these techniques. A comparative characterization of 17 flint maize landraces was carried out using RAPD markers by Okumus (11). Fourteen primers giving reliable and consistent polymorphic bands amplified 125 fragments (89%) with an average of 8.90 fragments per primer. The similarity was in range from 0.05 to 0.88. According to the observed results, Srdic *et al*. (15) reported a total of 68 RAPD fragments of different molecular weight were scored, 81% were polymorphic and gave 3 to 11 fragments per primer. The GD ranged from 0.124 up to 0.674. Cluster analysis showed a clear grouping of lines into two main heterotic groups. In another study conducted by Vilela *et al*. (18) the determined level of polymorphism was of 89.25%. The level of polymorphism is 61.8% in local waxy maize accessions and 88.3% in local normal maize accessions found with the aid of RAPD markers from 10 primers (7). Similarity values and polymorphic index allowed authors to distinguish 18 groups of maize accessions with distinctive genetic profiles. The molecular data grouped the waxy maize accessions into 11 main clusters and normal maize into 7 groups. Leal ***et al.*** (6) quantified genetic diversity among inbred lines originating from different soil and climate adaptations by using both RAPD and SSR markers to evaluate how well these two types of markers discriminated the popcorn genotypes. The used nine RAPD primers yielded 126 bands, of which 104 (82.53%) were classified as polymorphic, giving an average of 11.6% polymorphisms per primer. Meanwhile, 22 of theses bands were monomorphic (17.47%).

Correlation between genetic distances obtained through RAPD and SSR markers was relatively high (0.54). Mukharib ***et al.*** (9) detected a high level of polymorphism of 73.02% among the genotypes, as they used RAPD marker to assess genetic diversity in a selected group of maize inbred lines. The maximum detected genetic distance was 29.7%, and the minimum was 12.8%. RAPD markers were used by Shah ***et al***. (14) to characterize 10 commonly cultivated maize genotypes. A molecular marker-based phenogram led to the grouping of all genotypes into four major clusters, some of which were distantly related. The clusters showed a range containing from a single to multiple genotypes in a cluster. Authors observed a range of genetic similarities ranged from 60 to 100%.Yadav and Singh (19) used a set of maize inbred lines to compare how morphological, physiological characterization and RAPD molecular marker described variety relationships. The authors stated that all the inbred lines were confirmed as morphologically and physiologically distinct. From RAPD, random primers provide polymorphic amplification products and the genetic distance varying from 0.42 to 0.65. The study, therefore, represents an opportunity to investigate the role of selection by selfing in derivation of version lines from their base populations, and to estimate the magnitude of genetic and agronomic variations in the performance of cms populations per se and their ability to form elite crosses.

MATERIALS AND METHODS

To assess the contributions of selection and selfing in changing gene frequencies and genetic distinctness, eighteen of cms maize (***Zea mays*** L.) populations were grown for six seasons during 2008-2010 at the experimental farm of Crop Science Dept./College of Agriculture-Univ. of Baghdad. Maize lines were classified into three groups six lines of each, depending on its involving ***cms*** and ***rf*** genes: A, B and R, all were in the fifth generation (S5). Based on some desirable traits, selection was conducted on single plants of R-lines and B-lines. After three cycles of selection original and subpopulations have been evaluated at both, morphological and molecular levels.

Total genomic DNA extraction

The molecular genetic analysis were conducted in Medicinal and Molecular Biotechnology Dept./Center of Biotechnology/Al-Nahrain Univ. Seed samples were ground into a fine powder prepared for DNA extraction by using “wizard genomic DNA kit”.

DNA Quantification and Purity

The following formulas were used to estimate DNA concentration and purity ratio, respectively:

- $DNA\ concentration\ (\mu g/ml) = O.D.\ 260nm \times 50 \times Dilution\ factor$
- $DNA\ purity\ ratio = O.D.\mathbf{260}/O.D.\mathbf{280}$

Depending on DNA concentration, samples were diluted to a final concentration of 50 ng/µl for amplification.

Primers

Ten of decanucleotide primers from C, D, E and P series were used, obtained from (Alpha DNA-Canada) in a lyophilized form. Primers were dissolved in sterile distilled deionized water to get final concentration of (10pmol/µl) as provider instructed. Detailed description of the primers and their sequence are given in table (1).

Table(1):DNA primers used for random amplification of maize genomic DNA.

DNA primers code	*Sequence 5'→3'*
OPC-01	TTCGAGCCAG
OPC-02	GTGAGGCGTC
OPC-07	GTCCCGACGA
OPC-08	TGGACCGGTG
OPC-09	CTCACCGTCC
OPD-03	GTCGCCGTCA
OPD-05	TGAGCGGACA
OPD-08	GTGTGCCCCA
OPE-07	AGATGCAGCC
OPP-01	GTAGCACTCC

PCR (Polymerase Chain Reaction

Ready master mix (Go *Taq*®Green) has been used, which is composed of PCR buffer (2x, PH=8.5), dNTPs (400mM) and Taq polymerase (5 units). DNA amplification was carried out in PCR tubes containing 25µl reaction mixture composed of 12.5µl master mix, 2µl RAPD primer, 2µl DNA and volume was completed with D.D.W. The DNA was added to the reaction mixture and mixed thoroughly by gentle pipetting, then centrifuged mildly to collect all constituents and were subjected to the thermal profile detailed in table(2). Agarose Gel Electrophoresis: The DNA amplified fragments were separated by using agarose gel with concentration of 1.5% in 1X TBE buffer (54g of Tris-Base, 27.5g of Boric acid and 20ml of 0.5M EDTA (pH=8.0)). Electrophoresis was done until the tracking dye (Bromophenol blue) reached the edge of gel. Staining step with ethidium bromide (1gm of ethidium bromide/100ml sterile D.W.) for the agarose gel was of 25-35 minutes long, thereafter UV transilluminater was used to visualize DNA bands at wavelength of 365nm (8). Finally, the observed bands were photographed by gel documentation system.

Table (2): Program of RAPD-PCR

Steps	**Temperature ^{o}C**	**Time (min)**	**No. of cycles**
Initial Denaturtion	95	4	20 cycle
Annealing	36	1	
Extension	72	2	
Denatruation	95	1	15 cycle
Annealing	36	1	
Extension	72	1	
Denaturaion	95	1/2	10 cycles
Annealing	**36**	**1**	
Final Extension	**72**	**5**	

Analysis of Amplified Fragments Data

Regarding the previously known of DNA fragments weight of the marker (11497-216 bp), the molecular weight of the amplified DNA fragments was estimated with aid of Photo-Capture computer software (Consort-Belgium). For analysis genetic diversity, every scorable band was considered as single allele/locus and was scored as present (1) or absent (0).

Genetic similarity was calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) procedures as described by Nei and Li's (10):

- Genetic Similarity (GS) = 2Nij/(Ni+Nj)

Where: *Gs=Genetic Similarity between two populations.*

Nij = Total number of loci (bands) in populations i and j.

Ni = Total number of loci (bands) in population i.

Nj = Total number of loci (bands) in population j.

Polymorphism was estimated according to the following formula:

- Polymorphism % = (Np/Nt)×100

Where: *Np=the number of polymorphic bands of primer.*

Nt= the total number of bands of same primer.

RESULTS AND DISCUSSION

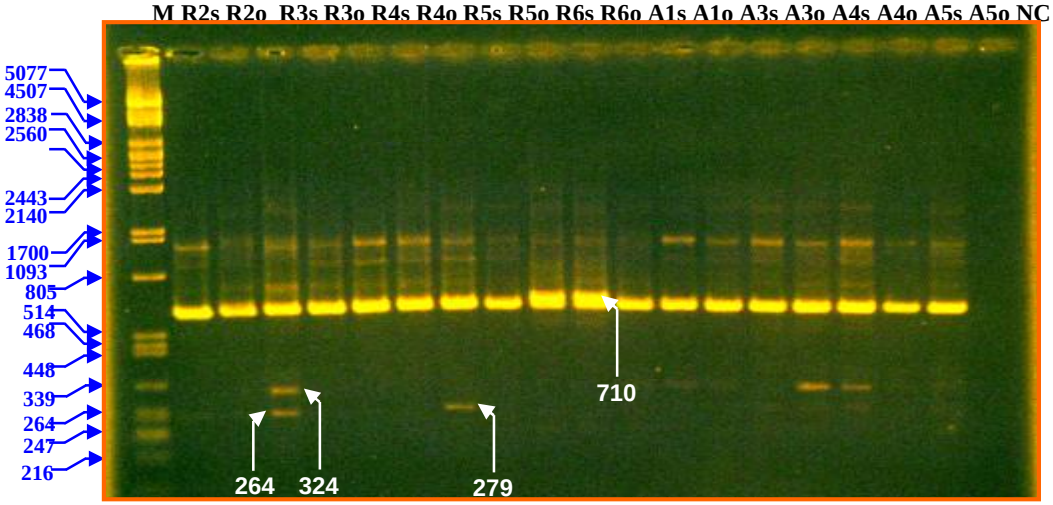
Analysis of Amplified Fragments

Genomic DNAs from maize populations were amplified using 10 primers. The results were listed in table 3. The present findings are further strengthened by previous reports presented by Okumus (11) and Srdic *et al*. (15). RAPD polymorphisms occurs due to base substitutions at the primer binding sites which prevents stable association with the primer, or structural rearrangements within the amplified sequence such as insertions, deletions and inversions.

Table(3):Scorable DNA fragments generated by 10 RAPD decamer primers through PCR, polymorphic fragments and percent polymorphism of maize cms populations.

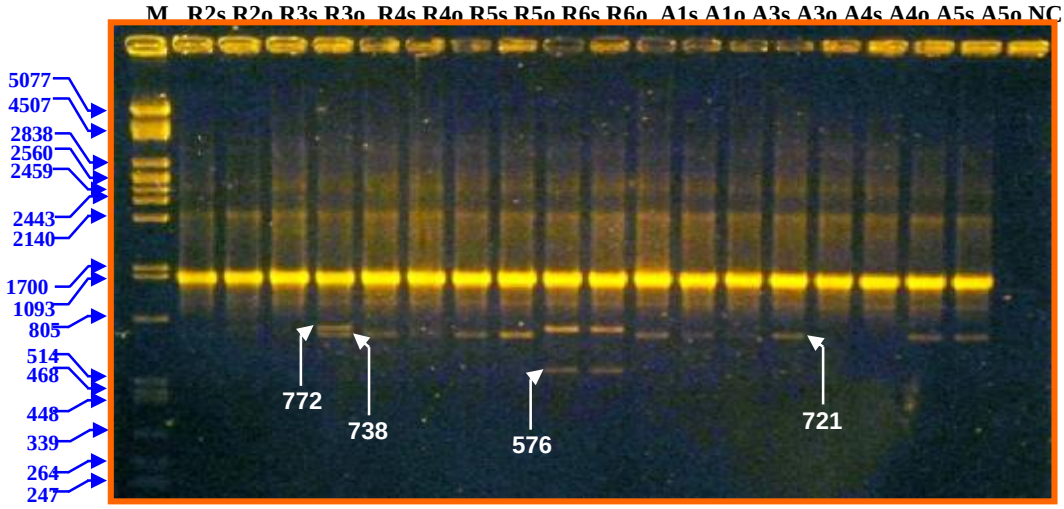
RAPD primers	*Total number of fragments*	*No. of polymorphic fragments*	*Percent of polymorphism (%)*	*Size of fragments (bp)*
OPC-01	8	7	88	264-920
OPC-02	6	3	50	576-2484
OPC-07	21	17	81	269-2717
OPC-08	14	13	93	339-2333
OPC-09	11	10	91	432-2315
OPD-03	13	11	85	337-2484
OPD-05	17	16	94	358-2250
OPD-08	15	8	53	314-2465
OPE-07	21	15	71	266-2250
OPP-01	13	9	69	380-2188
Total	139	109	78	264-2717

Primer OPC-01 figure(1) gave a total number of 8 fragments, 7 of them were polymorphic scoring polymorphism percentage of 88%. The previous reports presented by Vilela *et al*. (18) were supportive to these results. The molecular sizes of produced fragments ranged from (920bp) to (264bp). The primer produced unique fragments distinguished between some of the original and their corresponding subpopulations. One of these fragments was in R2S at the molecular size of (920bp). Based on the used primer, alteration with nucleotide sequences were resulted in two unique fragments show up in R3S at the molecular size of (324bp) and (264bp). Each of R5S and A1o presented 1 fragment at the molecular size of (279bp) and (920bp). Comparison between all entries indicated the distinctness of R6O population and its progeny R6S, as they showed a unique fragment at the molecular size of (710bp).



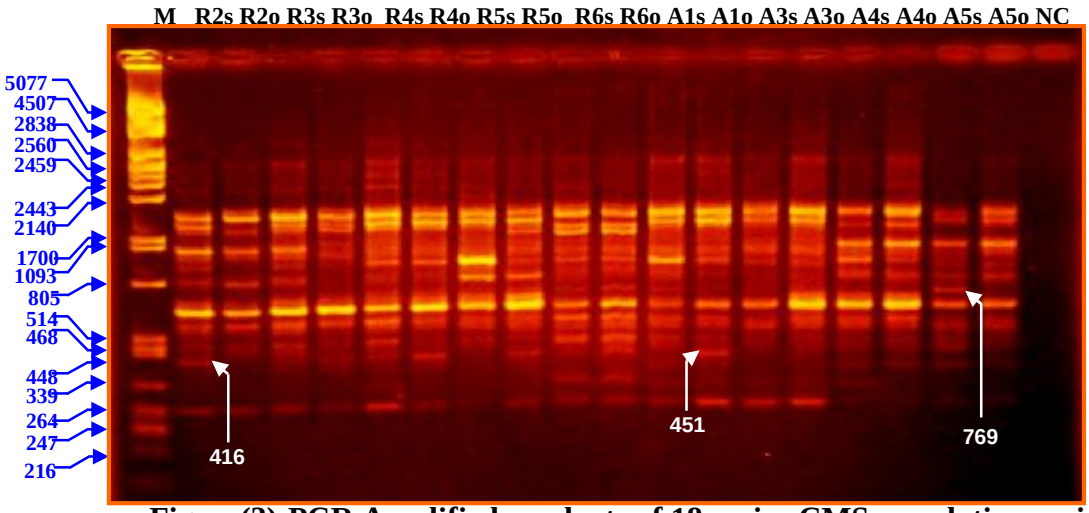
Figure(1):PCR Amplified products of 18 maize CMS populations using RAPD primer OPC-01.
M= Molecular marker (11,497bp ladder). NC=Negative Control

Six fragments were generated from OPC-02 primer figure(2), half of them were polymorphic. Hence, this primer showed the lowest percentage of polymorphism (50%). The amplified products ranged in molecular size between (2484bp) and (576bp). Monomorphic fragments among all the maize populations expressed the common genetic relationship among them, while polymorphic fragments expressed the genetic diversity. The primer produced unique fragmenting patterns allowing the discrimination of original against their descends. Two unique fragments presented in R3O at the molecular size of (772bp) and (738bp). Meanwhile 1 fragment with a molecular size of (721bp) was detected in A3s population. A6O showed a unique fragment at the molecular size of (1028bp) figure(11). The unique fragmenting pattern of OPC-02 primer in R6 resulted in 1 unique fragment at the molecular size of (576bp), which represented a verification of purity and genetic diversity of this populations.



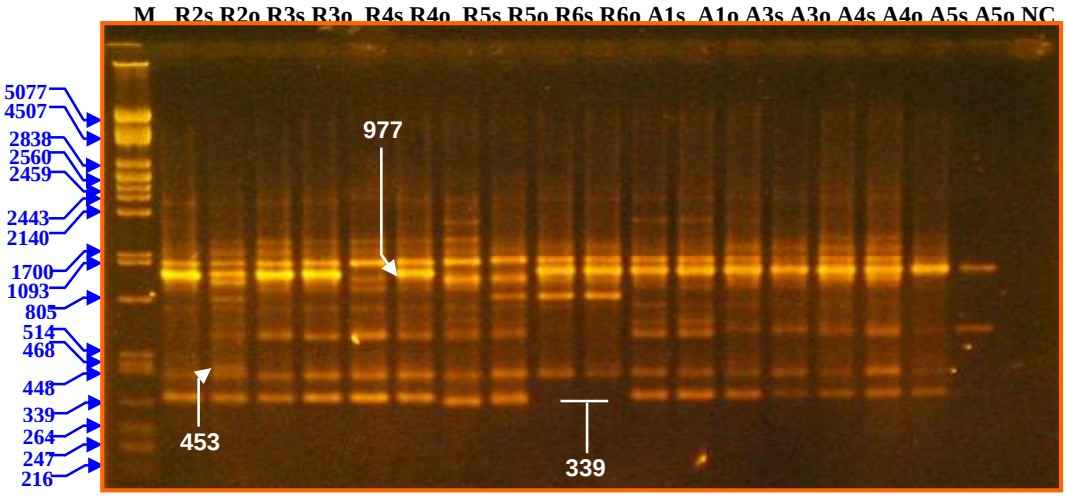
Figure(2):PCR Amplified products of 18 maize CMS populations using RAPD primer OPC-02.
M= Molecular marker (11,497bp ladder). NC=Negative Control

Primer OPC-07 scored one of the highest numbers of amplified fragments (21 fragment) in all studied populations figure(3). These fragments ranged in molecular size between (2717bp) and (269bp). Seventeen out of the total number of scorable fragments were polymorphic, indicated polymorphism percentage of (81%). Similar findings were reported by Srdic *et al.*(15) and Leal *et al*. (6). Three unique fragments obtained from using OPC-07 primer can be easily detected in R2S corresponding its ancestor with the molecular size of (2359bp), (930bp) and (416bp). Another fragment was detected in R3S at the molecular size of (796bp). A1S lost one fragment at the molecular size of (451bp) makes its existences in the original population unique. A5S and A6S populations gained new annealing loci as they owned unique fragments compared with their ancestors at the molecular size of (769bp) and (943bp), respectively. Likewise, the used primer resulted in fragment at the molecular size of (352bp) confirmed the genetic dissimilarity of R6 population compared with all entries in one hand, and on the other hand the less heterozygousity of this population based on same fragmenting pattern between the ancestor and its descend progeny.



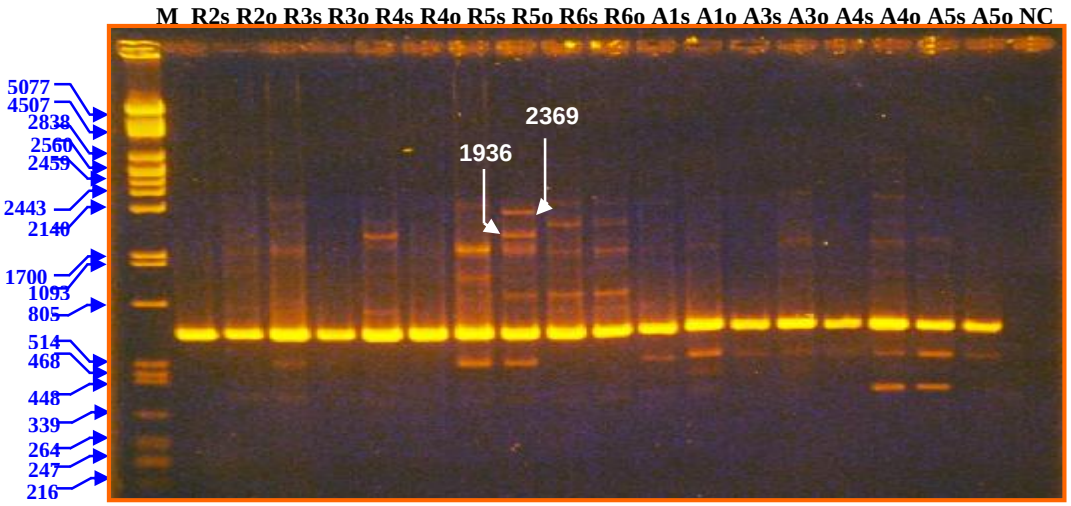
Figure(3):PCR Amplified products of 18 maize CMS populations using RAPD primer OPC-07.
M= Molecular marker (11,497bp ladder). NC=Negative Control.

A total of 14 fragments were produced by OPC-08 primer figure (4). As 13 out of the total fragment number were polymorphic, a high value of polymorphism percentage was reported (93%). The molecular sizes of amplified fragments were ranged from (2333bp) to (339bp). Fragmenting pattern obtained from using OPC-08 primer was discriminative to changes in allele frequency between original and subpopulations. The presence of four different fragments in R2O and its absence in R2S confirmed it's uniquely molecular size of (917bp), (781bp), (592bp), and (453bp). From figure (4), it can be noticed that R4S lost main annealing loci with this primer resulting in the absence of the main fragment, which can be easily detected in original R4 at the molecular size of (997bp). The changes in the annealing loci between the original and their descended subpopulations could be explained partly by the observations that *Ac* insertion alleles of a locus can give rise directly to *Ds*-like mutations at the same locus suggested that *Ds* and *Ac* elements were structurally related, and once *Ac* or *Ds* are methylated, their transposition is prevented. This would be the most direct mechanism by which DNA methylation blocks transposase action (13). R5S gained two unique fragments at the molecular size of (2361bp) and (2553bp), and lost one at the molecular size of (831bp). Absence of fragment with molecular size of (339bp) in both R6S and R6O populations supported their genetic distinctness and purity.



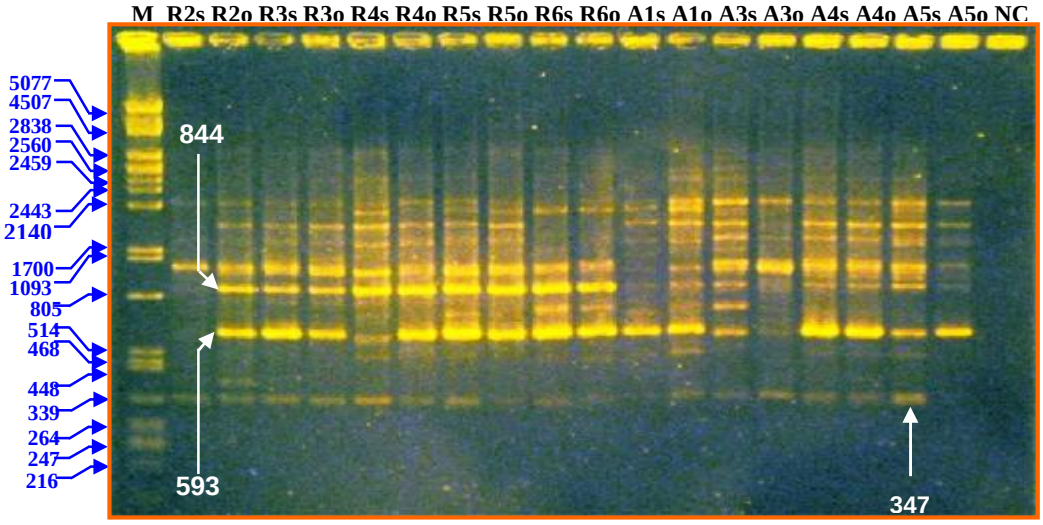
Figure(4):PCR Amplified products of 18 maize CMS populations using RAPD primer OPC-08.
M= Molecular marker (11,497 bp ladder). NC=Negative Control.

OPC-09 primer produced a total of 11 fragments figure(5), 10 of them were polymorphic, so computed percentage of polymorphism was (91%). Fragments molecular size ranged from (2315bp) to (432bp). Unique fragments were observed, three of them can be seen in R3S at the molecular size of (2140bp), (1950bp) and (523bp). Also, R4s showed three unique fragments at the molecular size of (2369bp), (2137bp) and (771bp). Through selection, R5S lost two of detectable fragments have the molecular size of (2369bp) and (877bp), at the same time the previously mentioned population gained two new fragments with the molecular size of (2207bp) and (989bp). The latest fragment seems to be fingerprint for R5S. Three unique fragments were noticed in A4O at the molecular size of (2338bp), (2266bp) and (428bp). A5S possessed new annealing site approved by the presence of unique fragment at the molecular size of (432bp).



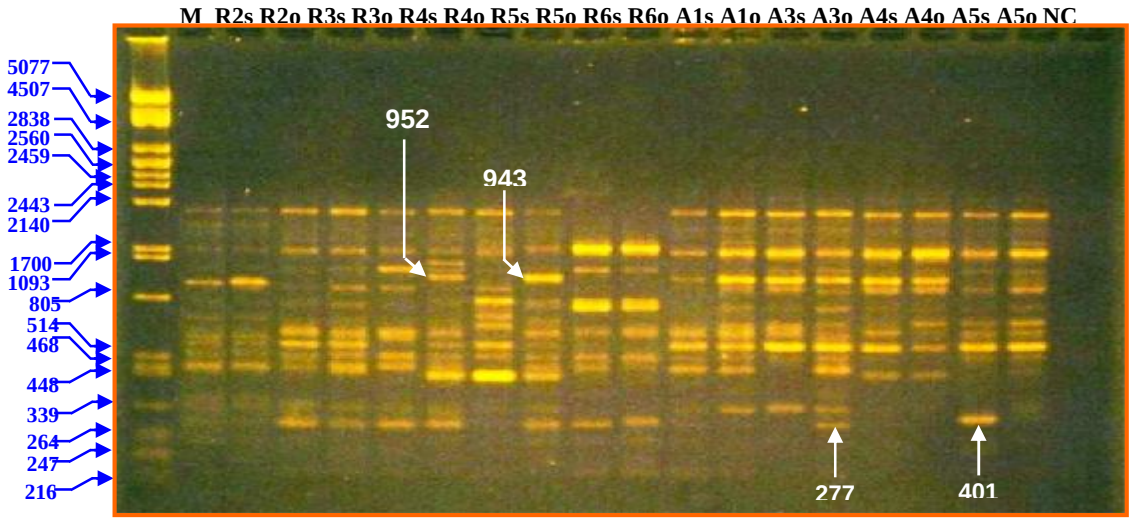
Figure(5):PCR Amplified products of 18 maize CMS populations using RAPD primer OPC-09.
M= Molecular marker (11,497bp ladder). NC=Negative Control.

OPD-03 primer gave thirteen scorable fragments figure(6), eleven of them were polymorphic scoring polymorphism percentage of (85%). These results agreed with those detected by Liet and Thinh (7) stated similar results too as they used local Vietnam maize accessions. Maximal molecular size of amplified fragment was (2484bp), while the minimum was of (337bp). Unique fragments were easily detected; four were in R2O at the molecular size of (2402bp), (844bp), (593bp) and (407bp). R4S gained a unique fragment at the molecular size of (2229bp). Another three unique fragments distinguished A5S from it's original population at the molecular size of (2229bp), (499bp) and (347bp). A6S gained a unique fragment regarding its ancestor at the molecular size of (2225bp) figure (12).



Figure(6):PCR Amplified products of 18 maize CMS populations using RAPD primer OPD-03.
M= Molecular marker (11,497bp ladder). NC=Negative Control

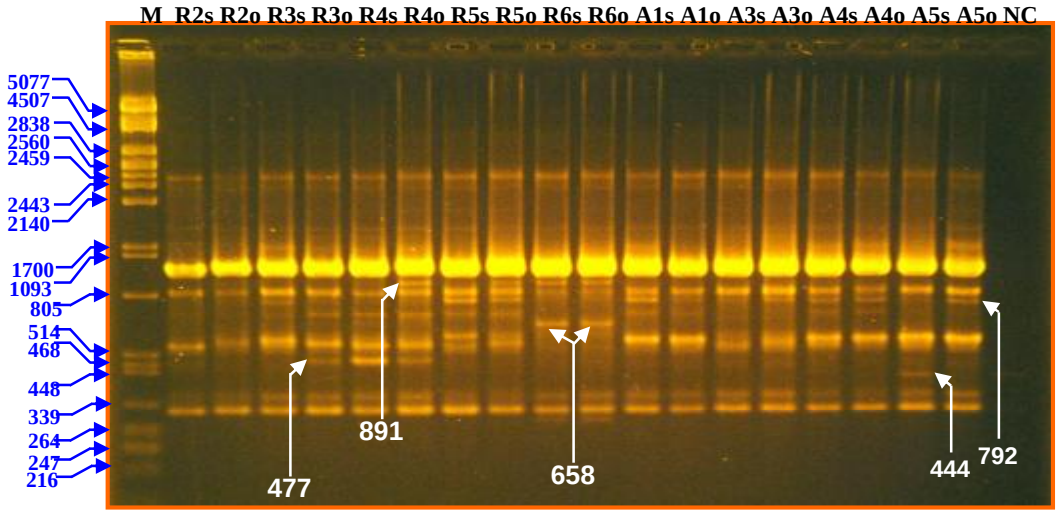
The results clearly indicated that primer OPD-05 figure(7)owned the highest percentage of polymorphism of (94%). The primer produced a total of 17fragments across 20 studied populations with molecular size ranged from (2250bp) to (358bp). Sixteen of these fragments were polymorphic. The primer had an excellent ability to distinguish between original and subpopulations, as it produced 1 unique fragment in R3O at the molecular size of (624bp). Four of the unique fragments in R4O were illustrated in figure(7)at the molecular size of (1063bp), (952bp), (551bp) and (469bp). Selection resulted in creating two new annealing sites in R5S at the molecular size of (868bp) and (708bp), meanwhile and for the same reason, R5S lost 3 fragments which can be easily located in R5O at the molecular size of (943bp), (612bp) and (481bp). A1O has 2 unique fragments at the molecular size of (756bp) and (435bp). Three of the unique fragments were obtained in A3O at the molecular size of (742bp), (472bp) and (377bp), whereas A4O and A5S showed just 1 fragment in at the molecular sizes of (661bp) and (401bp), respectively. A6S showed two unique fragments at the molecular size of (1950bp) and (904bp) figure(12). The most interesting pattern of fragmenting was showed by R6O and its progeny, which showed a unique fragment at the molecular size of (742bp). Two important things may affect the final number of amplified DNA fragments corresponding any primer, the first is the genetic heterozygosity of the studied populations, and the second is the severity and longevity of selection. Both of these factors have major effect on allele frequency, which in turn determining the frequency of annealing sites.



Figure(7):PCR Amplified products of 18 maize CMS populations using RAPD primer OPD-05.
M= Molecular marker (11,497 bp ladder). NC=Negative Control.

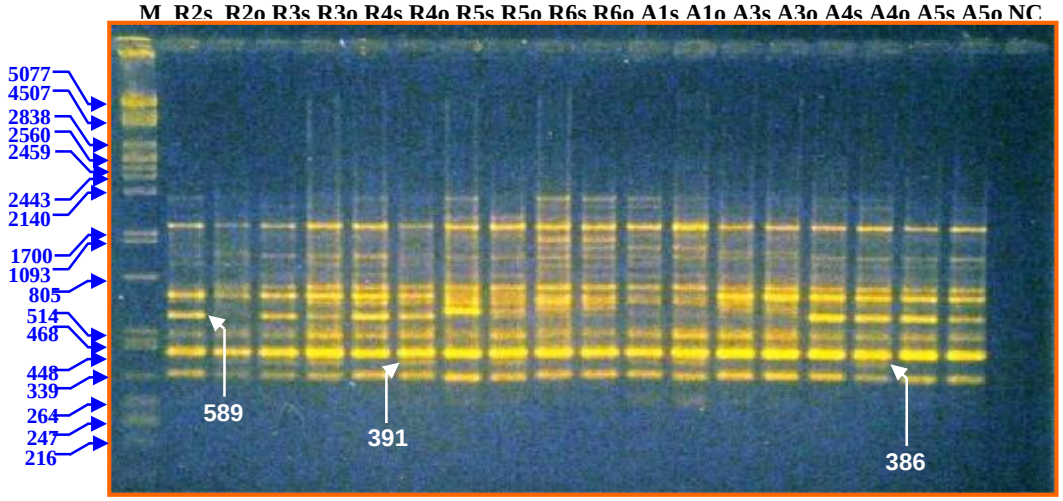
OPD-08 primer presented a total of 15 fragments figure(8), only 8 fragments found to be polymorphic. Therefore, percentage of polymorphism was too low (53%). Produced fragments with respect molecular size ranged between (2465bp) and (314bp). The previous mentioned primer was efficient in detecting unique fragments characterized either original or subpopulations. Two of these original fragments in R3O at the molecular size of (707bp) and (477bp) seem to be lost after selection in R3S, in the same time the latest gained new fragment with the molecular size of (774bp).

Absence of one fragment at the molecular size of 891bp in R4S made its presence in R4O unique. The same fragment disappeared in R5S (891bp), while R5O still possessed it. In case of the populations that descend from single seed, there is a big chance to lose some alleles especially those which were in heterozygous form and selection by selfing was performed. These alleles may provide annealing loci in the base population, and losing it will result in absence of special DNA fragment. These words may explain the absence of one fragment in A5S at the molecular size of (792bp), and the presence of a unique fragment in the same population at the molecular size of (444bp). R6 was conserved its unique genetic pool through selection process and showed special pattern of fragmenting confirmed by its unique fragments with the molecular size of (658bp) and (275bp).



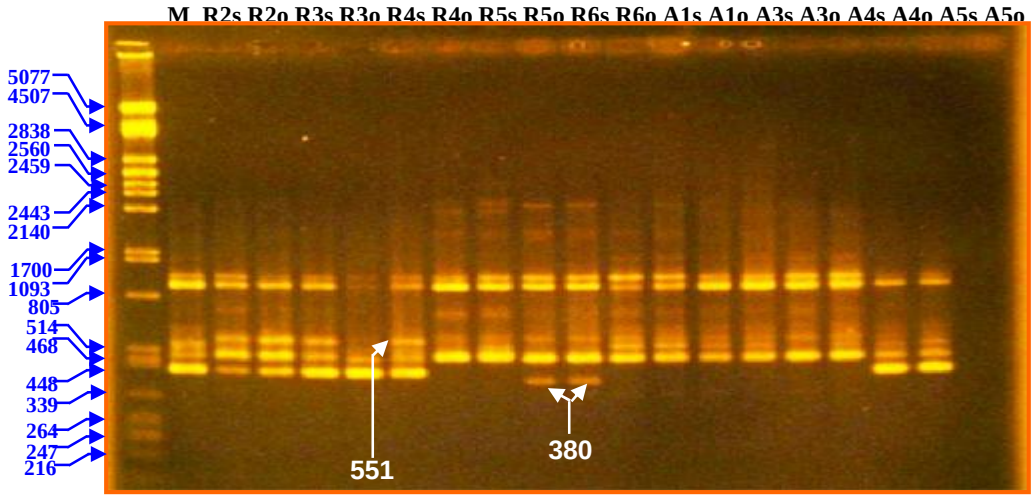
Figure(8):PCR Amplified products of 18 maize CMS populations using RAPD primer OPD-08.
M= Molecular marker (11,497 bp ladder). NC=Negative Control.

OPE-07 gave the highest number of scorable fragments (21) figure(9), (15) of them being polymorphic. The computed level of polymorphism was (71%). Mukharib *et al*. (9) documented similar results. The molecular size of fragments was in range from (2250bp) to (266bp). After three cycles of selection, R2S lost and gained annealing sites at the molecular sizes of (395bp) and (589bp), respectively. R4S didn't go so far as it lost and gained two different annealing sites at the molecular sizes of (391bp) and (965bp). The alterations in the annealing loci may be resulted from the transposition of *Ac/Ds*, which is in general for maize DNA transposable elements, occurs conservatively by a "cut and paste", non replicative mechanism, and is accompanied by its disappearance from the donor location and reintegration into a new site of the genome (13).Unique fragment can be recognized in R5S at the molecular size of (2276bp). As A4S lost one fragment, its ancestor still gains it at the molecular size of (386bp). Genetic diversity of R6 populations still shows up through presenting a unique fragment at the molecular size of (1080bp).

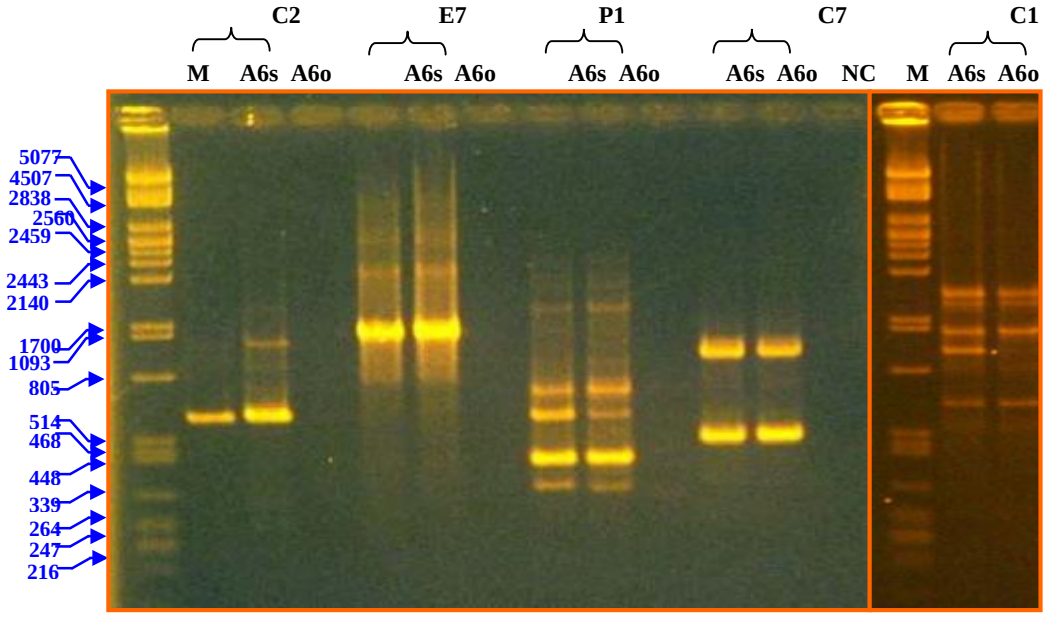


Figure(9):PCR Amplified products of 18 maize CMS populations using RAPD primer OPE-07.
M= Molecular marker (11,497bp ladder). NC=Negative Control.

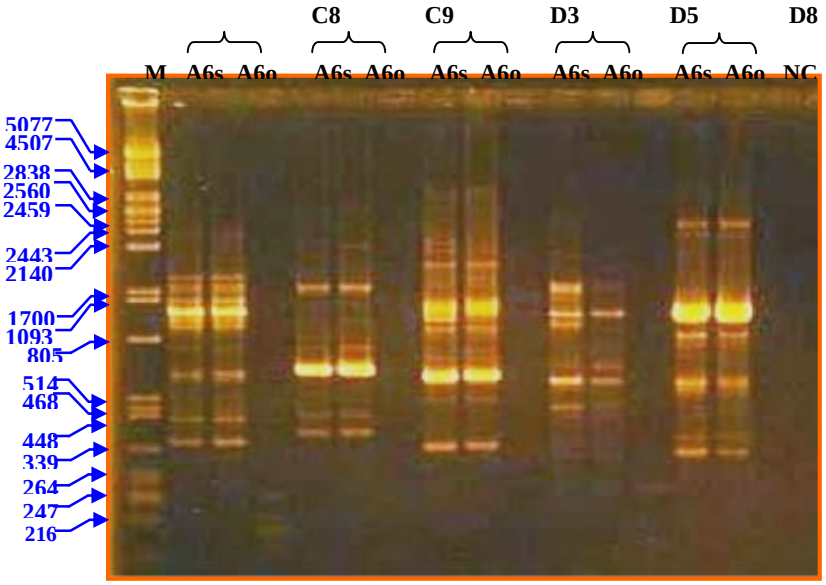
OPP-01 presented a total number of 13 fragments, 9 of them were found to be polymorphic figure(12). Polymorphism percentage was relatively low (69%) compared with rest of the primers. Amplified fragments ranged in molecular size between (2188bp) and (380bp). The primer showed fragmenting pattern which can aid in were discrimination of subpopulations against its ancestors. Both R2O and R4O distinguished from their descends in one fragment at the molecular sizes of (713bp) and (551bp), respectively. Each of R5S and A4S lost one fragment make it presence in their ancestors unique at the molecular sizes of (2188bp) and (539bp), respectively. There is a fact that in the presence of an *Ac* element, the *Ds* element can be transposed "jump" elsewhere in the chromosome. This transposition can cause a chromosome break at the point of insertion, so that the *Ds* element and any downstream loci are lost (13). Therefore, *Ac/Ds* elements may play an important role in modifying annealing sequences in maize DNA. Big magnitude of genetic dissimilarity approved by the two unique fragments which can be noticed in R6 populations at the molecular size of (380bp).



Figure(10):PCR Amplified products of 18 maize CMS populations using RAPD primer OPP-01.
M= Molecular marker (11,497 bp ladder). NC=Negative Control.



Figure(11):PCR Amplified products of 2 maize CMS populations using RAPD primers OPC-01, OPC-02, OPE-07, OPP-01 and OPC-07.
M= Molecular marker (11,497 bp ladder). NC=Negative Control.

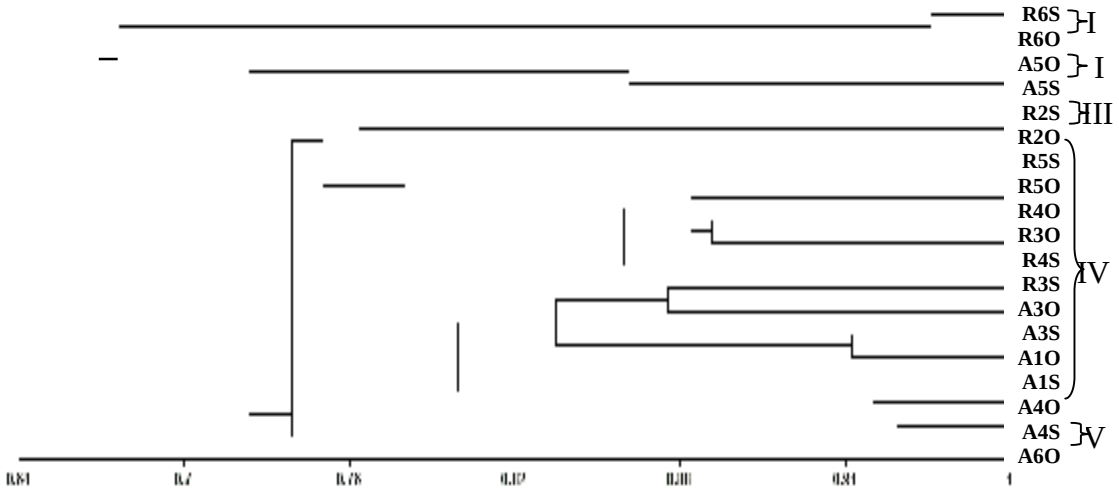


Figure(12):PCR Amplified products of 2 maize CMS populations using RAPD primers OPC-08, OPC-09, OPD-03, OPD-05 and OPD-08. M= Molecular marker (11,497 bp ladder). NC=Negative Control.

Genetic Similarity and Phylogenetic Analysis

Data matrix was constructed by comparing RAPD profiles on the basis of the presence (1) or absence (0) of each DNA fragment for each primer on the photographed agarose gels. The bivariate (1-0) data and genetic similarity (GS) coefficient matrices of twenty maize populations based on the data of 10 RAPD primers table(4) were used to construct a dendrogram by using unweighted pair group method with arithmetic average (UPGMA) through MVSP (Multi Variate Statistical Package) computer software. figures(13,14,15). Results from genetic similarity matrices generated by using Nei and Li's (1979) coefficients table(4), indicated as expected, a high level of similarity between subpopulations and their ancestors populations, with some exceptions. The highest genetic similarity (0.97) was observed between original and subpopulation of R6, while the lowest genetic similarity (0.76) was observed between original and subpopulation of R2. High and low genetic similarity detected by Carvalho *et al*. (3) supported these findings. Although subpopulations descended from original ones by selfing, there is considerable modification in the genetic loci at least those match the used primers. These results supported by the constructed phylogenetic tree, showed different position occupied by original and subpopulation as they analyzed separately figure(14,15). High and low estimates of GS between these populations indicated the efficiency of selection and selfing in making significant alterations with nucleotide sequence, which in turn affect the homology between the primer and the pairing site on the DNA strand, and finally the amplified DNA fragments.

The highest genetic similarity of (0.89) was observed between R3o and R4 original and subpopulations, meanwhile genetic distance widened slightly in R6 population after three cycles of selection as R6s possessed the lowest GS value of (0.57) against A5o. Okumus (11) and Shah *et al*. (14) had reported similar findings. The dendrogram figure(13) grouped the entire studied populations into five major clusters (I, II, III, IV and V). Each of the first four clusters was comprised of pairs of original and their descents subpopulations, whereas the fourth cluster was the largest and can be divided into two main subclusters. Cluster I was comprised of R6s and R6o; cluster II was comprised of A5o and A5s; cluster III was comprised of R2S and R2o; cluster IV was comprised of R5S, R5o, R4o, R3o, R4s, R3s, A3o, A3s, A1o, A1s, A4o and A4s; while cluster V was comprised of A6o and A6s. Surprisingly, phylogenetic tree showed that R3s and R4s were separated from their counterpart ancestors due to selection effect, and new genetic relationship was settled by grouping R3o and R4s populations in one cluster figure(16). As populations grouped in the same cluster, these populations expected to have common background. Surprisingly R3s and R3o were grouped in different clusters, although the first was pedigree of the second.



Nie and Li's

Figure(13):Dendrogram obtained by cluster analysis of 20 maize cms populations based on fragment polymorphisms generated by 10 RAPD primers.

Findings showed a range of genetic similarities among the original populations table(5), which ranged from (0.89) between R3o and R4o to (0.60) between A5o and R6o. Close genetic relationship was also reported by Mukharib *et al.* (9) and Yadav and Singh (19). The maximum GS estimate among subpopulations table(6) was (0.84) between R3s and R4s; A1s and A3s; A4s and R3s populations. Whereas minimum GS estimate (0.59) which revealed highly genetic diversity was observed between A5S and R6S.

Table(4):Average estimates of genetic similarity at DNA level among maize cms original and subpopulations using 10 RAPD primers.

Pop.	R2S	R2O	R3S	R3O	R4S	R4O	R5S	R5O	R6S	R6O	A1S	A1O	A3S	A3O	A4S	A4O	A5S	A5O	A6S	A6O
R2S	1.00																			
R2O	0.76	1.00																		
R3S	0.77	0.81	1.00																	
R3O	0.77	0.78	0.87	1.00																
R4S	0.78	0.75	0.84	0.89	1.00															
R4O	0.80	0.80	0.87	0.89	0.88	1.00														
R5S	0.72	0.74	0.81	0.79	0.78	0.79	1.00													
R5O	0.67	0.75	0.79	0.78	0.77	0.81	0.84	1.00												
R6S	0.63	0.64	0.70	0.69	0.71	0.71	0.69	0.72	1.00											
R6O	0.64	0.66	0.72	0.70	0.73	0.70	0.69	0.73	0.97	1.00										
A1S	0.75	0.76	0.81	0.80	0.78	0.79	0.79	0.78	0.68	0.71	1.00									
A1O	0.72	0.71	0.81	0.78	0.77	0.77	0.78	0.78	0.70	0.73	0.94	1.00								
A3S	0.76	0.73	0.81	0.79	0.79	0.80	0.78	0.74	0.67	0.69	0.84	0.84	1.00							
A3O	0.77	0.71	0.83	0.82	0.80	0.79	0.77	0.76	0.70	0.72	0.84	0.82	0.88	1.00						
A4S	0.74	0.76	0.84	0.79	0.78	0.76	0.80	0.73	0.62	0.65	0.82	0.79	0.80	0.80	1.00					
A4O	0.73	0.76	0.86	0.79	0.77	0.78	0.83	0.74	0.64	0.67	0.81	0.80	0.78	0.80	0.95	1.00				
A5S	0.69	0.68	0.77	0.72	0.74	0.71	0.67	0.70	0.59	0.61	0.73	0.74	0.75	0.74	0.73	0.73	1.00			
A5O	0.72	0.67	0.77	0.76	0.72	0.72	0.68	0.67	0.57	0.60	0.72	0.74	0.74	0.77	0.74	0.74	0.86	1.00		
A6S	0.68	0.74	0.77	0.71	0.71	0.74	0.71	0.70	0.68	0.68	0.77	0.74	0.76	0.74	0.75	0.76	0.75	0.70	1.00	
A6O	0.69	0.75	0.79	0.74	0.73	0.76	0.74	0.71	0.70	0.70	0.75	0.74	0.74	0.75	0.74	0.79	0.74	0.73	0.96	1.00

These results approved the divergent origin of A5 and R6 populations as they still owned thminimal GS value in both original and subpopulations. In general, there is acceptable agreement between genetic diversity and hybrid vigor, with some exceptions. Such results of Dhliwayo *et al*. (4) was supportive, as they demonstrated that genetic distances were not all ways significantly associated with hybrid performance, specific combining ability effects, or hybrid vigor in maize.

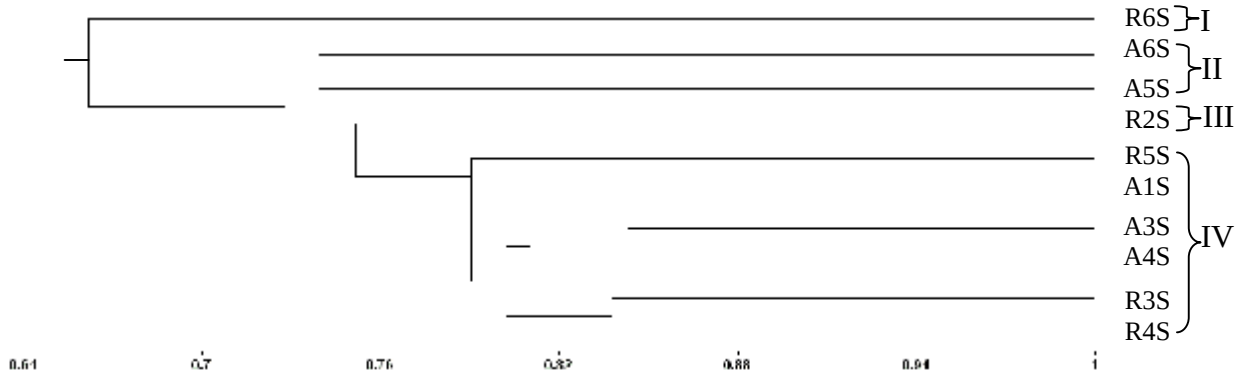
Table(5):Average estimates of genetic similarity at DNA level among 10 original populations of maize cms using 10 RAPD primers.

Pop.	R2O	R3O	R4O	R5O	R6O	A1O	A3O	A4O	A5O	A6O
R2O	1.00									
R3O	0.78	1.00								
R4O	0.80	0.89	1.00							
R5O	0.74	0.77	0.80	1.00						
R6O	0.67	0.71	0.71	0.73	1.00					
A1O	0.71	0.78	0.77	0.78	0.74	1.00				
A3O	0.71	0.82	0.79	0.76	0.73	0.82	1.00			
A4O	0.76	0.79	0.78	0.74	0.67	0.80	0.80	1.00		
A5O	0.67	0.76	0.72	0.68	0.60	0.74	0.77	0.74	1.00	
A6O	0.75	0.74	0.76	0.71	0.70	0.74	0.75	0.79	0.73	1.00

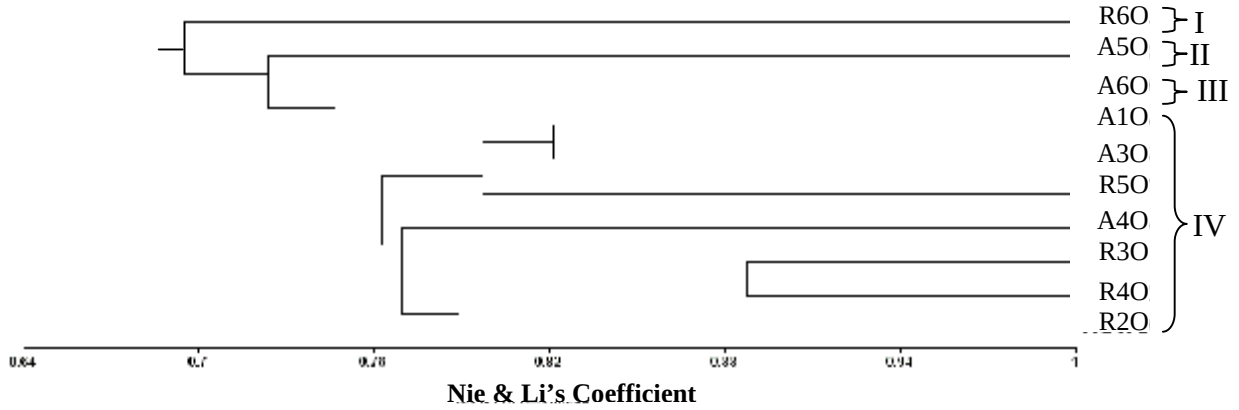
Table(6):Average estimates of genetic similarity at DNA level among 10 subpopulations of maize cms using 10 RAPD primers.

Pop.	R2S	R3S	R4S	R5S	R6S	A1S	A3S	A4S	A5S	A6S
R2S	1.00									
R3S	0.77	1.00								
R4S	0.78	0.84	1.00							
R5S	0.72	0.81	0.78	1.00						
R6S	0.63	0.70	0.71	0.69	1.00					
A1S	0.75	0.81	0.78	0.79	0.68	1.00				
A3S	0.76	0.81	0.79	0.78	0.67	0.84	1.00			
A4S	0.74	0.84	0.78	0.80	0.62	0.82	0.80	1.00		
A5S	0.69	0.77	0.74	0.67	0.59	0.73	0.75	0.73	1.00	
A6S	0.68	0.77	0.71	0.71	0.68	0.77	0.76	0.75	0.75	1.00

A comparison was made between original and subpopulations by separating from each other to clarify the possible changes in the genetic relationship and phylogenetic analysis after three cycles of selection. In general, the phenogram corresponds to the genetic similarity estimates and indicated variations between original and subpopulations that may be attributed to the selection role in losing some loci which were heterozygous in original populations. Also, individuals which have been selected are a critical step in developing new genetic materials. The phylogenetic tree figure(14) which was generated by using a similarity matrix grouped subpopulations into four major clusters. The clusters showed a range containing from a single to multiple populations in a cluster. The major clusters in the phylogenetic tree were subdivided into several sub-groups, which in turn comprised of single, pairs and multiple populations. After three cycles of selection, some populations revealed major alterations corresponding produced amplicones, which its pattern determines the cluster shape and the comprised populations. Other populations showed highly genetic stability and conserved its distinctness and occupied position in the phylogenetic tree, such as R6 figure(17), which seems to be descended from different origin. One of the important rules that determine the response magnitude of selection is the genetic heterozygosity of populations. So, there is always an expectation that selection can change the genetic composition of the heterozygous population more than the homozygous one. Selection had resulted in increasing genetic diversity between R3o and R4o populations; meanwhile genetic diversity was decreased between A3s and A1s populations, although these populations still grouped together. These results provide a clear view about the role of natural selection in evolution and arising of plant populations. Therefore, molecular markers are a predominant tool in investigating the genetic relationship between even close related populations.



Figure(14):Dendrogram obtained by cluster analysis of 10 maize cms subpopulations based on fragment polymorphisms generated by ten RAPD primers.



Figure(15):Dendrogram obtained by cluster analysis of 10 maize cms original populations based on fragment polymorphisms generated by ten RAPD primers.

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