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IMPACT OF HYBRIDIZATION ON THE PATTERN OF MOLECULAR GENETIC VARIATION IN MAIZE (*Zea mays*)

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KEYWORDS

Maize

Hybridization

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ABSTRACT

Present study was conducted to analyze the impact of hybridization on the molecular variation of maize. Total ten RAPD markers were used to assess the genetic diversity in hybridized maize crop and its relationships with their respective F1 hybrids was also established in present study. Among these, five were succeeding to amplify DNA fragments through PCR technique and produced total 31 fragments. Out of these thirty one, twenty two showed polymorphism and mean of these polymorphic percent scored 64.5%. Least genetic similarity was reported in L2×T1 against other genotypes; on the other hand L2×T2 and L3×T1 became closely related genera and gained 100 percent genetic similarity. Generally, the F1 hybrids acted in a different way compared with their corresponded parental lines, and the latest tend to occupy separate cluster in Dendrogram tree as they generally gained low values of genetic similarity. Meanwhile F1 hybrids showed high level genetic similarity among themselves. Result of present study revealed a clear impact of hybridization on the genetic diversity among maize genotypes and could play an important role in developing new genotypes.

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1 Introduction

Still we haven't understand many phenomena surrounding to us, hybrid vigor that feed millions of people are one of the phenomena that still undergo mystery until the moments even at the molecular level. Researchers are still working on making a better understanding on the mechanism of heterosis action and tracking the genetic heterogeneity from parents during its transition which in turn affect the performance of hybrids (Alfalahi et al., 2012).

Many of the previous studies addressed the supposed influence of genetic divergence between the parents on the strength of the hybrids, but most of those studies relied on superficial indicators to judge the presence or absence of genetic heterogeneity. It is well known that the morphology of the plant is largely affected by environmental fluctuations, which is reflected on the accuracy of the collected data (Bardakci, 2001; Gratani, 2014). The scientific scene witnessed the using of new molecular biology techniques and large numbers of highly informative DNA markers in order to develop some distinguishing differences between genetic variants regardless of the environmental impacts. Accordingly, from last few years random amplified polymorphic DNA (RAPD) technique became most commonly used molecular markers in evaluating molecular variations even within the same species.

According to Liet & Thinh (2009) RAPD marker is an attractive method for the analysis of genetic diversity among maize accessions and they detected that level of polymorphism reached 61.8% in local waxy maize accessions and 88.3% in local normal maize accessions by hybridization. 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. Furthermore, Leal et al. (2010) examined the efficiency of RAPD and SSR markers in discriminating among popcorn genotypes. The nine used RAPD primers yielded 126 bands, of which 104 (82.53%) were polymorphic and the recording polymorphisms average of 11.6% per primer. Authors concluded that both RAPD and SSR primers were efficient in evaluating genetic diversity in the evaluated genotypes, though RAPDs yielded more polymorphisms. Similarly, high level polymorphism (73.02%) was also reported by Mukharib et al. (2010), when they used RAPD marker to assess genetic diversity in a selected group of maize inbred lines. The maximum genetic distance was 29.7%, while the minimum observed genetic distance was 12.8%. Based on these results, authors believed that the RAPD markers can be successfully employed in identifying genetic diversity in maize inbred lines.

Shah et al. (2010) were grouped maize into four main groups. Among these some clusters contain single and some contain multiple genotypes and the genetic similarities ranged from 60 to 100%. Similarly, Yadav & Singh (2010) conducted a study

with the aim of assessing the role of RAPD molecular marker in establishing relationships among maize genotypes. According to these researchers all the inbred lines can be confirmed on the basis of morphologically and physiologically traits with the help of RAPD. The genetic distance varying from 42 to 65%, and close genetic relationship was detected among the used genotypes which turn resulted in close clusters formation.

Alfalahi et al. (2012) used RAPD markers to evaluate trends in genetic diversity among twenty cms line populations derived from early and advanced selection cycles. All the 10 RAPD primers used for initial screening were found polymorphic. As author mentioned, the genetic similarity was ranged from 50 to 94%. 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.

Mrutu (2015) also tried to assess the seed genetic purity of forty seven hybrid maize using random amplified polymorphic DNA markers and reported total 134 polymorphic bands which shows genetic similarity ranged from 0.09 to 0.99. Cluster analysis based on Jaccard's similarity coefficient grouped the genotypes into two major clusters; one cluster contained 42 genotypes and the other cluster contained five genotypes. Result of study concludes that the identical phenotypes were found to be genetically different, and with aid of RAPD markers 94 of these were found polymorphic and scoring 83.9 percent of polymorphism. Results of study revealed that only some pairs displayed genetic divergence and this may due to common ancestral parent (Hemavathy, 2015).

Further to all of the above, this study may be an addition to the previous efforts which have been made to assess the genetic diversity and relationships among maize germplasm collections using DNA markers. Moreover, an effort has been made to track genetic variation at the molecular level from parents to their F1 hybrids and impact of hybridization on the stability of the genetic relationship was also assessed by using phylogenetic analysis.

2 Materials and Methods

2.1 DNA Extraction

Total genomic DNA was extracted from maize seeds by using Genomic DNA Mini Kit-Plant (Geneaid Biotech Ltd.-Taiwan) and extraction was carried out by following instruction mentioned in the manufacture protocol.

2.2 Estimation of the quality and quantity of the isolated genomic DNA

The quality of total extracted genomic DNA was assessed by mixing 7µl of each DNA sample with 3µl of loading dye (0.25g Bromphenol blue dye; 30ml glycerol).

Table 1 Primers used for random amplification of maize genotypes.

<i>RAPD primers</i>	<i>Sequence</i>	<i>RAPD primers</i>	<i>Sequence</i>
<i>OPA-07</i>	5'-AAGTCCGCTC -3'	<i>OPD-20</i>	5'-TGTCATCCCC -3'
<i>OPA-09</i>	5'-GGGTAACGCC -3'	<i>OPI-01</i>	5'-AACCTGGCA-3'
<i>OPA-11</i>	5'-CAATCGCCGT -3'	<i>OPO-04</i>	5'-AACCTGGCA-3'
<i>OPA-13</i>	5'-CAGCACCCAC -3'	<i>OPN-16</i>	5'-CAAGGTGGGT -3'
<i>OPC-12</i>	5'-TGTCATCCCC -3'	<i>GB-08</i>	5'-GTCCACACGG -3'

This was followed by the running this mixed samples on 0.8% agarose gel submerged in 1×TBE (Tris base, Boric acid, EDTA) buffer. After 30 minutes gel was stained for 20 minutes with ethidium bromide in a dark place and visualized and photographed under UV light using White Film Camera (667).

DNA was quantified by checking the optical density (OD) at 260 and 280 nm wave length. The purity of genomic DNA was determined by the A260/A280 absorbance ratio. According to the quantity of extracted DNA, samples were diluted to prepared final concentration of 50ng/μl. DNA concentration was estimated through the following formula:

DNA concentration (μg/ml) = O.D. 260nm x 40 x Dilution factor

Ten RAPD primers of different series (GB, A, C, D and N) were obtained from Geneaid Biotech. Ltd., Taiwan in lyophilized form, and used for the polymorphism study (Table1). Primers were dissolved in sterile distilled deionized water to make the final concentration of (10pmol/μl) as per manufactured instruction.

Amplification reactions were carried out in 20 μl volume (5 μl DNA, 2 μl of primer, 1.5 μl NaCl, 6.5 μl of d.d. water added to 5 μl of ready master mix), Labnet thermal cycler (USA) was used to amplify PCR products with the recommended PCR thermoprofile viz. Initial denaturation was performed at 94°C for 5 min., 45 cycles of denaturation was followed at 94°C for 1 min., then primer annealing was done at 36°C for 1 min., finally extension step was for 2 min. at 72°C.

After amplification, 7 μl from each sample was mixed with 3 μl of loading dye (0.25g Bromphenol blue dye; 30ml glycerol) and run on 1.5% agarose gel submerged in 1×TBE (Tris base, Boric acid, EDTA) buffer, after 1 hour gel was stained with ethidium bromide in a dark place for 20 minutes. The gel was visualized and photographed under UV light using White Film Camera (667).

Genetic similarity was analyzed with aid of Popgene computer software (version 32). Every scorable band was considered as

single allele/locus and scored 1 for the presence of band and 0 for the absence (0). Genetic similarity was calculated by using Unweighted Pair Group of Arithmetic Means (UPGMA) procedures as described by Nei & Li (1979).

Genetic Similarity (GS) = $2N_{ij}/(N_i+N_j)$

Where: Gs=Genetic Similarity between two populations; N_{ij} = Total number of loci (bands) in populations i and j; N_i = Total number of loci (bands) in population i; N_j = Total number of loci (bands) in population j.

While the polymorphism was estimated according to the following formula (Alfalahi et al., 2012):

Polymorphism % = $(N_p/N_t) \times 100$

Where: N_p =the number of polymorphic bands of primer; N_t = the total number of bands of same primer.

3 Results and Discussion

3.1 Polymorphisms among the selected maize genotypes

All the used primers resulted in successful amplifications were polymorphic; hence, they proved to be very useful in resolving the genetic structure of the studied genotypes. The number of amplified fragments varied from 4 to 10 fragments with molecular size mean ranged from 141.2 bp to 321.6 bp (Table 2). The highest number of polymorphic fragments (8) was produced by the primer OPN-16, while the maximum percent of polymorphism (87.5%) was recorded with the primer OPC-12. These findings were agreed with the polymorphism detected by Leal et al. (2010). Primers efficiency between the used genotypes was varied from primer to primer and it depending on its ability to amplified unique fragment which could be used as finger print for related genotype. The percentages of polymorphism were ranged from 25 to 87.5% recording a mean of 64.4%. Similar type of findings was reported by Yadav & Singh (2010) and Mukharib et al. (2010). Some genotypes has specific fragment or band on the gel and these are the unique locus for that genotype, like fragment with molecular size of 200 bp which have been missed in L1, L2, T2 and T3 with the using of GB-08 primer (Figure 1).

The F1 hybrid L1×T3 gained a new annealing site with OPC-12 primer resulted in producing unique fragment with molecular size of 220 bp, meanwhile the same hybrid lost two annealing sites should produce fragments with molecular size ranged from 250 to 290 bp (Figure 1).

3.2 Phylogenetic Analysis

It was expected that each F1 hybrids will be genetically closest relative to both of his parents or at least one of them. It was expected because the genetic material of an individual is a combination of his parent's genetic materials and similar type of opinion also created for phenotypic characters. This general rule is not application without any exceptions, factors which are responsible for affecting this rules mutations, crossing over, transposable elements, in addition to the epigenetic effects like DNA methylation which may alters the individual performance without going through DNA sequence changes.

Genetic distance is a parameter which providing important insight into the differentiation pattern among genotypes. In present study it was persisted to separate the analysis of genetic relatedness of parents among F1 hybrids, and then joint

them together in one scheme in attempt to detect the impact of hybridization on the genetic relationships over generations.

Analysis of phylogram tree (Dendrogram) it can be observed that there is a clear impact of hybridization on the genetic divergence among the used genotypes (Fig. 2, 3 and 4). Here it was also reported that the F1 hybrids acted differently compared with their corresponded parental lines. Also, the parental lines occupied separate part in dendrogram tree which represented low values of genetic similarity while many of F1 hybrids genetically comes closed to each other which represented higher values of genetic similarity.

Maximal genetic similarity among the tested genotypes was 92% and it was reported between the genotype T1 and T2 (Table 3) while the minimal genetic similarities was recorded between the selected genotype L1 and L4 (70%). Results presented in table revealed that lines were genetically divergent more than testers which may be because of its genetic background. When this line compared against testers, it was reported that L1 and T2 showed the highest genetic similarity and it reached up to 91.9%. On the other hand, the minimal value of genetic similarity was 71.4% and 71.8% between L3 line and each of T1 and T2 testers respectively.

Table 2 Amplified and polymorphic DNA fragments generated by RAPD primers and percentage of polymorphism in selected maize genotypes.

RAPD primers	Total number of fragments	No. of polymorphic fragments	Percent of polymorphism (%)	Size of fragments (bp)
GB-08	4	1	25	172-320
OPA-09	4	2	50	130-340
OPC-12	8	7	87.5	110-612
OPD-20	5	4	80	140 -620
OPN-16	10	8	80	154 -310
Total	31	22	64.5	141.2 -440.4

Table 3 Genetic similarity values of seven parental maize lines with the used of five RAPD primers (OPA-09, OPC-12, OPD-20, OPN-16 and GB-08).

Genotypes	L1	L2	L3	L4	T1	T2	T3
L1	1.000						
L2	0.919	1.000					
L3	0.789	0.821	1.000				
L4	0.700	0.780	0.905	1.000			
T1	0.850	0.829	0.714	0.773	1.000		
T2	0.919	0.895	0.718	0.732	0.927	1.000	
T3	0.757	0.789	0.872	0.878	0.780	0.842	1.000

Here L1, L2, L3, L4 lines and T1,T2 and T3 testers with their respective top cross hybrids.

Figure 1 PCR Amplified products of 19 maize genotypes using four RAPD primers (GB-08 (1), OPA-9 (2), OPC-12 (3) and OPN-16(4) running on agaros gel (L1, L2, L3, L4 lines and T1,T2 and T3 testers with their respective top cross hybrids).

Figure 2 Constructed dendrogram of seven parental maize lines based on genetic similarity values with aid of five RAPD markers (OPA-09, OPC-12, OPD-20, OPN-16 and GB-08).

These results confirmed by the cluster analysis which separate parental genotypes into two main clusters I and II (Figure 2). The first cluster composed of two lines (L3 and L4) and one tester (T3). Whereas, the second cluster was composed of two sub-cluster, the first composed of T1 and T2 testers, while the second was composed of L1 and L2 lines.

In order to study the effect of hybridization process on the genetic relationship among genotypes pedigree, F1 hybrids which results from Line×Tester crossing system were genetically analyzed with aid of RAPD molecular markers. The values of GS among F1 hybrids (Table 4) showed clearly that hybridization played different role in the different genotypes. For example, surprisingly L2×T2 and L3×T1 were matched completely scoring 100% genetic similarity even they are not sharing the same parents, but in the same time T1 and T2 testers gained the highest GS value among parental genotypes this may explain the identical performance of their hybrids.

The high GS value between L4×T1 and L4×T3 hybrids was expected as they have L4 line participate in both of them, on the opposite direction L1×T3 hybrid choose to act differently with the lowest GS values (≈73%) and occupied one cluster alone.

Figure 4 is showing a combined dendrogram of all the tested genotypes altogether (Lines, Testers and F1 hybrids) in one (Fig. 4). F1 hybrids are genetically closet to their ancestor parents as expected, but this is not happened every time and some hybrids separate from their parents and constrain a new genetic relationship with different genotypes like L1×T2 which its DNA sequence related with the used primers become more identical with that of T1 tester scoring GS similarity reached 93.3%. The L2 line was succeeded in conservation of its family genetic relationship as it sustain the closet to its F1 hybrid L2×T3 with GS value of 95% (Table5).

Table 4 Genetic similarity values of 12 F1 maize hybrid with the used of five RAPD primers (OPA-09, OPC-12, OPD-20, OPN-16 and GB-08).

Genotypes	L1XT1	L1XT2	L1XT3	L2XT1	L2XT2	L2XT3	L3XT1	L3XT2	L3XT3	L4XT1	L4XT2	L4XT3
L1XT1	1.000											
L1XT2	0.810	1.000										
L1XT3	0.800	0.818	1.000									
L2XT1	0.773	0.833	0.783	1.000								
L2XT2	0.821	0.744	0.732	0.756	1.000							
L2XT3	0.850	0.818	0.810	0.783	0.927	1.000						
L3XT1	0.821	0.744	0.732	0.756	1.000	0.927	1.000					
L3XT2	0.800	0.864	0.810	0.826	0.829	0.905	0.829	1.000				
L3XT3	0.826	0.920	0.792	0.885	0.851	0.875	0.851	0.875	1.000			
L4XT1	0.878	0.800	0.791	0.809	0.952	0.930	0.952	0.884	0.898	1.000		
L4XT2	0.829	0.844	0.884	0.851	0.857	0.930	0.857	0.930	0.857	0.909	1.000	
L4XT3	0.884	0.851	0.800	0.857	0.909	0.933	0.909	0.889	0.941	0.957	0.913	1.000

Figure 3 Dendrogram of 12F1 maize hybrids based on genetic similarity values with aid of five RAPD markers (OPA-09, OPC-12, OPD-20, OPN-16 and GB-08).

It's clear that small group of genotypes occupied one sub-cluster consist of L3, L4, T3 and L1×T1 hybrid, while the rest of genotypes were occupied the other sub-cluster, except L1×T3 and L2×T1 hybrids which acts differently against each other and the rest of genotypes as they split up lonely. These findings are in accordance with the findings of Shah et al. (2010), which revealed that clusters showed a range containing from a single to multiple genotypes in a cluster, based on

genetic similarities started with 60% and ended with 100%. The results showed firmly that the genetic relationship could shift totally and new genetic relationship could be settled for many reasons such as crossing over process and Ac/Ds transposable elements. There is a need to use large number of RAPD DNA markers or SSR for more accuracy and get unbiased estimation for the role of hybridization in creating new genetic materials.

Figure 4 Constructed dendrogram of nineteen maize genotypes based on genetic similarity values with aid of five RAPD markers (OPA-09, OPC-12, OPD-20, OPN-16 and GB-08).

Genotypes	L1	L2	L3	L4	T1	T2	T3	L1XT1	L1XT2	L1XT3	L2XT1	L2XT2	L2XT3	L3XT1	L3XT2	L3XT3	L4XT1	L4XT2	L4XT3
L1	1.000																		
L2	0.919	1.000																	
L3	0.789	0.821	1.000																
L4	0.700	0.780	0.905	1.000															
T1	0.850	0.829	0.714	0.773	1.000														
T2	0.919	0.895	0.718	0.732	0.927	1.000													
T3	0.757	0.789	0.872	0.878	0.780	0.842	1.000												
L1XT1	0.811	0.842	0.769	0.780	0.829	0.895	0.895	1.000											
L1XT2	0.829	0.810	0.698	0.756	0.933	0.905	0.810	0.810	1.000										
L1XT3	0.769	0.850	0.683	0.698	0.837	0.850	0.750	0.800	0.818	1.000									
L2XT1	0.744	0.773	0.711	0.766	0.809	0.773	0.727	0.773	0.833	0.783	1.000								
L2XT2	0.895	0.872	0.850	0.762	0.762	0.821	0.769	0.821	0.744	0.732	0.756	1.000							
L2XT3	0.923	0.950	0.829	0.791	0.837	0.900	0.800	0.850	0.818	0.810	0.783	0.927	1.000						
L3XT1	0.895	0.872	0.850	0.762	0.762	0.821	0.769	0.821	0.744	0.732	0.756	1.000	0.927	1.000					
L3XT2	0.872	0.900	0.732	0.837	0.884	0.900	0.750	0.800	0.864	0.810	0.826	0.829	0.905	0.829	1.000				
L3XT3	0.800	0.826	0.809	0.857	0.857	0.826	0.826	0.826	0.920	0.792	0.885	0.851	0.875	0.851	0.875	1.000			
L4XT1	0.850	0.878	0.810	0.818	0.818	0.878	0.829	0.878	0.800	0.791	0.809	0.952	0.930	0.952	0.884	0.898	1.000		
L4XT2	0.900	0.927	0.762	0.773	0.864	0.927	0.780	0.829	0.844	0.884	0.851	0.857	0.930	0.857	0.930	0.857	0.909	1.000	
L4XT3	0.857	0.884	0.864	0.870	0.826	0.884	0.884	0.884	0.851	0.800	0.857	0.909	0.933	0.909	0.889	0.941	0.957	0.913	1.000

Table 5 Genetic similarity between the nineteen maize genotypes with the used of five RAPD primers (OPA-09, OPC-12, OPD-20, OPN-16 and GB-08).

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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