DETECTION OF GENETIC VARIABILITY IN *Zea mays* INBRED LINES USING SSRs AND SRAP MARKERS

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Maize (*Zea mays* L.) is the third most important cereal crop in the world after wheat and rice as well as in Egypt (FAO, 2015). According to the FAO report in 2015, the harvested area of maize in Egypt decreased from 1041,345 hectare to 900,000 in the years 2012 and 2013, respectively. On the other hand, the yield productivity was 77723 tons in 2012 while it decreased to 72222 tons in 2013.

Since maize cultivation has changed with the revolution in genetics and maize breeding programs, which was depend on characterization and genetic diversity among breeding material (i.e. inbred lines, hybrids, populations, landraces and races), so identification of genetically distant parental combinations provides best crop improvements for breeders (Elçi and Hançer, 2015). Also, the identification of new maize line with high potential of yield production can improve maize productivity in Egypt.

Molecular marker technology provides effective, fast, accurate and appropriate tools for crop improvement. For genotype characterization, various molecular markers (viz., RFLP, RAPD, SSR, AFLP etc.) possess many advantages over the morphological markers (Gupta *et al*., 1996).

Elçi and Hançer (2015) indicated that genetic differentiation of maize grains can be performed more accurately and efficiently using molecular markers. Where, these molecular markers would be more efficient, fast and cheap than grow-out test (GOT) at the field. Combining that, molecular markers now provide an excellent tool of obtaining large amounts of genetic data, where genetic information plays a significant role in determining the effectiveness of work in many areas (Heikal *et al*., 2007 and 2015**)**.

Among these markers, the Simple Sequence Repeats (SSRs) markers are of particular importance as these are PCR-based, highly polymorphic, reliable and reproducible (Gupta *et al*., 1996). The SSRs loci comprise of highly variable arrays of 2 to 6 base pair tandem repeats (Kassahun and Prasanna, 2003). Many

Egypt. J. Genet. Cytol., 44:291-307, July, 2015
Web Site (*www.esg.net.eg*)

SSRs markers are now available in maize under public domain facilitating their utilization for diverse purposes in genetics and plant breeding. SSRs have been used for varietal identification, seed purity testing, genetic similarity analysis and marker-assisted selection of crops in many species and can be easily detect both parental alleles because of their co-dominancy (Elçi and Hançer, 2015).

More recently, Li and Quiros (2001) developed and published information on the dominant marker technique SRAP. The sequence-related amplified polymorphism (SRAP) markers are used to amplify coding regions of DNA with primers targeting open reading frames. These markers proved to be robust and highly variable. Where, SRAP showed some advantages; the primers are common in different plants and do not need to be developed since beginning. The three selective bases (the first codon) at the 3\ end may be the random one of four bases (A, T, C, G), so the forward and reverse primers have 4^3 (64) possibilities, respectively. The forward and reverse primers were combined randomly to get 64^2 (4096) primer combinations, which reduce the cost for primers synthesis. Secondly, the PCR procedure of SRAP is simple and basically fixed which has no complex multiple steps such as digestion, ligation, re-amplification and selective amplification as AFLP analysis (Qiao *et al.*, 2007). SRAP markers have been used primarily for agronomic and horticultural purposes, developing quantitative trait loci in advanced hybrids and assessing genetic diversity of large germplasm collections (Robarts and Wolfe, 2014).

The objective of the present study was to detect genetic variability in three testers of white *Zea mays* and eight selfed inbred lines in regarding to the dry weight of 100 grains using SSRs and SRAP markers.

MATERIALS AND METHODS

A. Plant materials

In total, grains of eleven *Zea mays* genotypes including; three testers: Giza 1, Single cross 10 (S.C.10) and three ways cross 310 (T.W.C. 310) and eight white inbred lines *S1.19, S1.45, S1.46, S1.50, S1.51, S1.59, S1.61* and *S1.64.* These inbred lines which gave the highest yield with the previous testers were selfed and analyzed in this study. These selected inbred lines were derived throw selection from the population of Giza 2 as described in a previous treatise performed by El-Beially *et al.* (2007).

B. Methods

a. Dry weight of grains

The dry weight of 100 grains was measured and the means were estimated as described in a previous treatise performed by El-Beially *et al*. (2007).

b. DNA extraction

Bulk genomic DNA was extracted using 100 mg of grinded grains from each sample. DNA was extracted according to

the supplier's instructions using Gene JET^{TM}, plant genomic DNA purification mini kit (Fermentas). The extracts were stored at -20°C until used.

c. SSRs analysis

For each genotype , SSRs analysis was done on bulked DNA samples, using ten maize microsatellite markers (Table 1) which were selected according to their chromosomal location- from the maize genome data base (http//www. maizegdb.org). The PCR program was as follow: Initial denaturation at 94°C for 4 min for 1 cycle, followed by 35 cycles of template denaturation at 94°C for 1 min, primer annealing at 65 or 70°C for 50 sec. according to the used primer (Table 1), primer extension at 72°C for 2 min. and final extension at 72°C for 5 min. The SSRs reaction mixture with a total volume of 25 µl consisted of forward and reverse primers at 0.35 µM, 8 µl Maximo Taq DNA polymerase (GeneOn, Germany) which containing (dNTPs at 1.2 µM, Mg^{2+} at 32.0 µM, 0.8 U Taq DNA polymerase, PCR buffer (20 mM KCl, 16 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, pH8.8) and 50 ng of genomic DNA (El-Taher, 2011). DNA amplifications were performed with *Biometra T1 gradient Thermalcycler*. PCR products were run on 1.5% agarose gel electrophoresis, stained with ethedium bromide and visualized with UV transilluminator.

d. SRAP analysis

In this study, fifty SRAP primer combinations- using five forward primers and ten reverse primers- were applied for PCR amplification and analysis of 11 genotypes (Table 2). The SRAP-PCR reaction mixture with a total volume of 15 µl consisted of primers at 0.3 µM, 8 µl Maximo Taq DNA Polymerase (GeneOn, Germany) which containing (dNTPs at 1.2 µM, Mg^{2+} at 32.0 µM, 0.8 U Taq DNA polymerase, PCR buffer (20 mM KCl, 16 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, pH8.8) and 30 ng of genomic DNA. DNA amplifications were performed with *Biometra T1 gradient Thermalcycler*. SRAP PCR program was conducted as follow: 94°C for 5 min followed by 5 cycles of 94°C for 60 s, 35°C for 60 s, and 72°C for 60 s; and 35 cycles of 94°C for 60 s, 50°C for 60 s, 72°C for 60 s; and a final extension at 72°C for 7 min (Dawei *et al.*, 2010). The amplified products were electrophoresed in 2% ethidium bromide stained agarose gel (Liu *et al*., 2008) and visualized with UV transilluminator.

e. Data analysis

Phoretix electrophoresis gel image analysis, ID software was used for scanogram tracing of fragments size (bp). Data matrices were entered as (0/1) for absence/presence of fragments, respectively, into the NTSYS (Numerical Taxonomic and Multivariate Analysis System) program, version 2.1, Applied Biostatistics Inc. (Rohlf, 1998). Similarity coefficients were used to construct dendrograms using the UPGMA (Unweighted Pair Group Method with Arithmetic average) routing in the NTSYS software.

Correlations among the two obtained similarity matrices were performed using the Mantel's test (Mantel, 1967) calculated as the usual person correlation coefficient between two matrices, using XLSAT software version 1.03 (2015).

Polymorphism information contents (PIC) were calculated according to Anderson *et al*. (1993) using the following simplified formula: PICi = 1-Σ $0p^2ij$, Where pi is the frequency of the j^{th} allele for marker j^{th} summed across all alleles for the locus.

RESULTS AND DISCUSSION

a. Means of 100 grains dry weight

The weight means of 100 grains of the following *Zea mays* genotypes: three testers: Giza 1, Single cross 10 (S.C.10) and three ways cross 310 (T.W.C. 310) and eight white inbred lines *S1.19, S1.45, S1.46, S1.50, S1.51, S1.59, S1.61* and *S1.64*, were: 34.38, 22.80, 36.10, 29.51, 31.4, 27.30, 32.76, 27.60, 38.20, 27.2, 28.50 gram, respectively. This result was described in a previous treatise performed by El-Beially *et al.* (2007)

b. SSRs analysis

Ten SSRs loci distributed on 10 chromosomes (Table 1) were assayed in the studied eleven maize genotypes. The SSRs primers produced 24 alleles corresponding to an average of 2.4 per locus. The number of the amplified alleles per each SSRs varied from one (Phi015, Umc1152, Umc1014 and Phi064) to five (Phi021). Out of these ten primers, only six primers were polymorphic (Fig. 1 and Table 3). The profiles of these six SSRs exhibited different alleles per locus in the samples, with homozygous and heterozygous individuals clearly identifiable. The six polymorphic primers produced 18 polymorphic alleles showing an average of three alleles per locus. The SSRs markers showed alleles of different molecular sizes ranged from 82 to 355 bp (Table 3).

The number of heterozygosity detected for the six polymorphic SSRs loci ranged from one (0.45 heterozygosity) for Phi022 locus to 11 (1 heterozygosity) for Phi034, Phi022 and Phi021 loci (Table3). The homozygous genotypes according to the Phi085 marker results were *S1.50* and *S1.61*. The three testers and *S1.59* and *S1.61* were homozygous in the results of Umc2050 marker. While only S.C.10 tester was homozygous according to Phi0127 results.

The polymorphism information content (PIC) recorded by the six polymorphic SSRs markers ranged from 0.5 to 0.8 with an average of 0.64. The highest PIC recorded to Phi021 while the smallest was detected in both Umc2050 and Phi0127 (Table 3).

The results of the ten SSRs markers showed that six markers were found to be polymorphic. These six markers were used for testing seed homozygosity and variability analyses (Table 3). Despite the presence of selfed eight inbred lines, the observed high level of heterozygosity sus-

pected that a mixing occurred during pollination, harvesting or processing with other genotypes. These reasons are considered the main reasons for contamination in production (Salgado *et al*., 2006**)**. Also, the heterozygosity reveled by SSRs markers in the inbred lines could be the results of contamination by stray pollen during inbred development or due to mutation caused spontaneously or by transposable elements during inbred development as indicated by Jyoti Kumar and Prasanna (2005). On the other hand, their study indicated that PIC values that ranged from '0' (monomorphic) to '1' (highly discriminative with many alleles in equal frequencies consider discriminative power of the marker. So as indicated by Geth *et al.* (2002), it is necessary to take into consideration the source of the inbred lines if a high level of homozygosity is desired.

The dendrogram resulted from analyzed results of SSRs using UPGMA method showed that the maize inbred lines were separated from the three testers: Giza 1, S.C.10 and T.W.C. 310 (Fig. 2). According to the SSRs dendrogram, the maize genotypes were divided into two main clusters; each one contained two sub- clusters. All the testers were grouped together into a separated sub- cluster at the top of the dendrogram. The inbred lines *S1.19, 45* and *46* were grouped in the next sub- cluster. In the middle of the dendrogram, the *S1.50* inbred line was separated in a sub- cluster while the rest of the inbred lines were grouped in the rest of sub- clusters. The highest genetic similarity (G.S) was observed between the two testers S.C.10 and T.W.C. 310 with G.S of 0.78. For the inbred lines, the highest related inbred lines were *S1.51* and *S1.61* showing G.S of 0.72, followed by *S1.59* and *S1.61* with G.S of 0.70. On the other hand the most unrelated inbred lines were *S1.19* and *S1.61* where they had 0.48 G.S (Table 4).

UPGMA ordered the three testers first in a broad group then inbred lines genotypes. In regarding to the weight of dried 100 grains the SSRs markers did not order the genotypes in the dendrogram according to the weight on the contrary of the SRAP markers. Where, it gathered the high yielded *S1.59* inbred line with a moderate yielded inbred line S1.64 in one sub- cluster. Despite SSRs markers are of particular importance (Gupta *et al*., 1996). Our results showed partial congruent in profiling the inbred lines genotype regarding their 100 grains dry weight. These results are in confirmation with Jyoti Kumar and Prasanna (2005) whom indicated that grouping of ten genotypes on the basis of SSRs profiling was found to be partially congruent with their pedigree and breeding history. This could be due to various reasons including small number of SSRs loci analyzed in this study.

c. SRAP analysis

Sequence-related amplified polymorphism (SRAP) was used to detect the genetic variability among the eleven *Zea mays* genotypes. Fifty primer combina-

tions were used, 37 primer combinations (Table 5) gave stable and reproducible amplification patterns and the other combinations were rejected for failing to produce amplification or displaying monomorphic patterns. The 37 selected SRAP primer combinations generated 292 amplified fragments (Fig. 3) with an average of 7.9 fragments per primer combination, ranged from approximately 30-1313 bp in size, of which 156 (53.4%) were polymorphic (Table 5) with an average of 4.2 polymorphic fragments per primer. The highest percentage of polymorphism (100%) resulted using Me3- Em10 primer combination, whereas the lowest percentage (14.29%) was recorded using Me2- Em9 primer combination.

The number of reproducible and polymorphic fragments produced by each primer combination varied. Table (5) shows that 163 (55.8%) DNA amplified fragments were common fragments (monomorphic), while 44 (15%) fragments were markers (unique) in the eleven genotypes. The different SRAP primer combinations amplified the number of fragments ranged from 5 (Me1- Em1, Me1- Em8 and Me3- Em9) to 13 (Me1- Em7). The highest number of polymorphic fragments (17) produced by the primer combinations (Me2- Em1 and Me4- Em1) whereas the primer combination Me1- Em1 and Me2- Em9 generated the lowest number of amplified fragments (one).

The polymorphism information content (PIC) recorded by the 37 primer combinations of the SRAP markers ranged from 0.24 to 0.91 with an average of 0.68. The highest PIC value recorded to Me3- Em10 combination, while the lowest was detected in Me2- Em9 combination (Table 5).

The SRAP results analysis using UPGMA method was able to separate the maize lines from the three testers: Giza 1, S.C.10 and T.W.C. 310 (Fig. 4). The SRAP dendrogram was divided into two distinct clusters; the first contained two sub-clusters. The first sub-cluster contained two groups, the first one contained the three testers and the second group contained *S1.64* line. The second sub- cluster contained *S1.51* and *S1.61* inbred lines. The second cluster contained the rest of the inbred lines. The most related inbred lines according to SRAP genetic similarity (G.S) analysis were the inbred lines S1.50 and *S1.45* with G.S of 0.84 followed by *S1.46* and *S1.19* with G.S of 0.74. The most unrelated genotypes were T.W.C 310 and *S1.59* where they showed genetic similarity of 0.11 (Table 6).

SRAP analytic system was set up and successfully used in the identification of the genetic variability for the studied 11 genotypes. The dendrogram constructed by the SRAP combination results had ordered the genotypes in relation to their dried 100 grains weight which means that this marker is more accurate than the SSRs marker but it is difficult in determining the heterozygous genotypes from homozygous ones. Guo *et al.* (2012) observed that similar to other markers scored as dominant, SRAP amplicons cannot yield heterozygosity descriptors.

d. Correlation among the two markers using Mantel test

Mantel's test (Mantel, 1967) was used to determine the correlation between SSRs and SRAP molecular markers. There was a moderately high correlation between the molecular similarity matrices. The correlation between SSRs and SRAP markers was 0.68, indicating that booth techniques are efficient for evaluating genetic diversity in the maize genotypes.

e. Genetic similarity and phylogenetic relationship based on combined SSRs and SRAP data

The genetic similarity and relationships among the eleven Egyptian *Zea mays* genotypes were estimated using combined data of SSRs and SRAP results. The results showed that the highest similarity index; 78% was observed between T.W.C. 310 and S.C.10 genotypes, while the lowest similarity index (46%) was between *S1.61* and *S1.19* genotypes (Table 7). The dendrogram for phylogentic relationships revealed that there are two main clusters. The first one had two sub-clusters where, the three testers Giza1, S.C.10 and T.W.C. 310 genotypes were grouped in a separate sub-cluster distinct from the other genotypes, while the second sub- cluster of that cluster had grouped *S1.19, S1.45, S1.46* and *S1.50* inbred lines. The rest of the inbred lines (*S1.51, S1.61, S1.59* and *S1.64*) were grouped in the second cluster (Fig. 5).

In this study, ten SSRs primers and fifty SRAP combinations were applied with eleven *Zea mays* genotypes to detect the genetic variability between them regarding the dry weight of 100 grains. The dendrogram results of both SSRs and SRAP separately and their combination had separated the three testers in one sub-cluster from the inbred lines; indicating that these three genotypes are so related to each other (the G.S ranged from 0.69 to 0.78 for SSRs results, 0.68 to 0.79 for SRAP results and 0.69 to 0.78 for combination results) showing an obvious genetically distance from the other studied inbred lines (Tables 4, 6 and 7). Falahati-Anbaran *et al.* (2007) indicated that greater genetic variation could be useful to make a synthetic variety in order to select a population with a higher genetic distance to reach more heterosis. That reason could explain why these three testers in our study gave the highest results with the studied eight inbred lines as described in a previous treatise performed by El-Beially *et al.* (2007).

The combining results of SRAP and SSRs markers gave a slight different dendrogram. Where it had only three sub-clusters and ordered the inbred lines in different way from SRAP, but in relation to SSRs results which showed partially congruent with the dried 100 grains weight. Since SRAP targets ORF and SSRs targets simple sequence repeats, also most SRAP markers are dominant while SSRs are co-dominant, our results indicated that gathering the two markers results to achieve a dendrogram could be partial congruent in profiling the studied genotypes. This could be due to limiting the

average number of alleles identified per Simple Sequence Repeats (SSRs) locus during screening the studied genotypes which led to higher observed genetic similarity than was by SRAP markers. This is in contrast to Qiao *et al.* (2007) who stated that a genetic map with high-intensity which reflects the distribution of both ORFs and SSRs could be constructed when the two marker techniques are used together.

SRAP markers have demonstrated their utility by elucidating greater levels of variation within groups of highly related individuals when used in conjunction with other markers for comparative purposes, including RAPD (Comlekcioglu *et al.*, 2010), ISSR (Zhang *et al*., 2012), SSRs (Guo *et al.*, 2012) or AFLP (Youssef *et al*., 2011) and have proven their utility by elucidating greater levels of variation between examined individuals (Robarts and Wolfe, 2014).

So, it can be concluded that, seed genetic variability analysis and differentiation of the maize inbred lines in regarding to the dry weight of 100 grains, can be performed more accurately and efficiently using the SRAP markers than SSRs markers. So, the use of SRAP markers should be viewed analogous to morphological character states, which can be used in the delimitation and assessment of variation within and between individuals. While the SSRs markers are good for discrimination heterozygosity which will be helpful for hybrid maize seed industry and could be more powerful in genetic profiling when using an efficient number of markers.

SUMMERY

Information on germplasm variability and relationships among elite materials is fundamentally important in crop improvement. In this study, genetic variability of 11 maize genotypes, three testers [Giza 1, Single cross 10 (S.C.10) and Three ways cross 310 (T.W.C. 310)] and eight selfed inbred lines (*S1.19, S1.45, S1.46, S1.50, S1.51, S1.59, S1.61* and *S1.64*) was tested using ten microsatellite (SSRs) loci distributed on 10 chromosomes of maize, and 50 SRAP marker combinations, regarding the means of dry weight of 100 grains. For the SSRs results, only six markers were polymorphic. A total of 24 alleles were detected among the maize genotypes. At each locus, the number of alleles varied from one to five, with an average of 2.1 alleles. On the basis of the genetic similarity coefficients, the SSRs UPGMA clustering analysis separated the genotypes into two clusters showing four groups. The most unrelated genotypes were *S1.19* and *S1.61* where they had 0.48 genetic similarities. The PIC ranged from 0.5 to 0.8 with an average of 0.31. The SRAP results gained 37 polymorphic primers combinations with polymorphism average of 53.4%. The most related genotypes were inbred lines *S1.50* and *S1.45* with genetic similarity of 0.84. The PIC ranged from 0.24 to 0.91 with an average of 0.68. The combined results of both SSRs and SRAP create a dendrogram with three groups.

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Table: (1): Information of the SSRs primers and their sequence.

SSRs primer	Chromosome no.	Forward and reverse Primer sequence	Annealing Temp.
phi064	1	F:3'CCGAATTGAAATAGCTGCGAGAACCT5' R:3'ACAATGAACGGTGGTTATCAACACGC5'	70°C
phi127	2	F: 3'ATATGCATTGCCTGGAACTGGAAGGA 5' R:3'AATTCAAACACGCCTCCCGAGTGT5'	70°C
umc2050	3	F: 3'CTCCTGCTGTGATTCTAGGACGA5' R:3'CTGGATCTCGGCATGGTCTT5'	70°C
phi021	4	3'TTCCATTCTCGTGTTCTTGGAGTGGTCCA5' R: 3'CTTGATCACCTTTCCTGCTGTCGCCA5'	70°C
phi085	5	F: 3'AGCAGAACGGCAAGGGCTACT5' R: 3'TTTGGCACACCACGACGA5	65°C
umc1014	6	F: 3'GAAAGTCGATCGAGAGACCCTG5' R: 3'CCCTCTCTTCACCCCTTCCTT5'	65°C
phi034	7	F: 3'TAGCGACAGGATGGCCTCTTCT5' R: 3'GGGGAGCACGCCTTCGTTCT5'	65°C
phi015	8	F: 3'GCAACGTACCGTACCTTTCCGA5' R: 3'ACGCTGCATTCAATTACCGGGAAG5'	65°C
phi022	9	F: 3'TGCGCACCAGCGACTGACC 5' R: 3'GCGGGCGACGCTTCCAAAC5'	65°C
umc1152	10	F:3'CCGAAGATAACCAAACAATAATAGTAGG 5' R: 3'ACTGTACGCCTCCCCTTCTC5'	65°C

Table (2): Forward and reverse SRAP primers information.

Name	Forward primer	Name	Reverse primer
Me1	TGA GTC CAA ACC GGA TA	Em1	GAC TGC GTA CGA ATT AAT
Me2	TGA GTC CAA ACC GGA GC	Em2	GAC TGC GTA CGA ATT TGC
Me3	TGA GTC CAA ACC GGA AT	Em3	GAC TGC GTA CGA ATT GAC
Me 4	TGA GTC CAA ACC GGA CC	Em4	GAC TGC GTA CGA ATT TGA
Me5	TGA GTC CAA ACC GGA AG	Em5	GAC TGC GTA CGA ATT AAC
		Em6	GAC TGC GTA CGA ATT GCA
		Em7	GAC TGC GTA CGA ATT CAA
		Em8	GAC TGC GTA CGA ATT CAC
		Em9	GAC TGC GTA CGA ATT CAG
		Em10	GAC TGC GTA CGA ATT CAT

Table (3): The allele's size range, number, polymorphism, heterozygosity information and the PIC obtained by ten SSRs markers.

SSRs marker	Allelic range size (bp)	Number of alleles	Polymorphic alleles	Major allele frequency	No. of hetero-zygotes	Value of heterozygosity Per locus	Homozygous genotypes	PIC
Phi034	82-205	4	3	1	11	1	-	0.75
Phi015	122	1	-	1	0	0	-	-
Phi085	201-235	3	3	0.54	9	0.82	*S1.50 -S1.61*	0.67
Phi022	123-355	4	4	0.63	11	1	-	0.75
Umc1152	891	1	-	1	0	0	-	-
Umc1014	941	1	-	1	0	0	-	-
Umc2050	93-120	2	1	1	6	0.55	Giza 1 (T.W.C. 310) (S.C.10)- *S1.59 - S1.61*	0.50
Phi064	98	1	-	1	0	0	-	-
Phi0127	87-114	2	2	0.72	10	0.91	(S.C.10)	0.50
Phi021	114-140	5	5	0.63	11	1		0.80
Total		24	18					
Average		2.4	3					0.64

∗PIC: Polymorphism information contents

Table (4): The SSRs genetic similarity (GS) for the three testers and eight *Zea mays* inbred lines.

	Giza1	S.C.10	T.W.C. 310	S1.19	S1.45	S1.46	S1.50	S1.51	S1.59	S1.61
S.C.10	0.69									
T.W.C. 310	0.70	0.78								
S1.19	0.63	0.65	0.62							
S1.45	0.59	0.57	0.62	0.65						
S1.46	0.56	0.52	0.57	0.67	0.61					
S1.50	0.54	0.60	0.64	0.56	0.59	0.67				
S1.51	0.53	0.62	0.59	0.61	0.61	0.66	0.63			
S1.59	0.53	0.59	0.58	0.58	0.59	0.57	0.65	0.67		
S1.61	0.52	0.58	0.57	0.48	0.57	0.59	0.65	0.72	0.70	
S1.64	0.55	0.57	0.57	0.55	0.51	0.57	0.59	0.62	0.68	0.68

Table (5): The total fragments generated, polymorphism information and PIC using 37 SRAP combinations.

Primer combination	Total fragments	Fragments size range (bp)	Polymorphic fragments	Monomorphic fragments	Marker (unique) fragments	Percentage of polymorphism	PIC
Me1- Em1	5	122-691	1	4	4	20.00	0.32
Me1- Em2	9	177-666	5	4	4	55.60	0.76
Me1- Em4	11	138-945	7	4	2	63.64	0.80
Me1- Em5	7	192-675	4	3	2	57.14	0.70
Me1- Em6	9	141-665	6	3	2	66.67	0.82
Me1- Em7	13	157-948	6	7	3	46.15	0.69
Me1- Em8	5	71-657	2	3	0	40.00	0.56
Me1- Em9	10	93-1099	6	4	4	60.00	0.78
Me1- Em10	7	100-659	5	2	0	71.43	0.82
Me2- Em1	10	99-1313	7	3	2	70.00	0.84
Me2- Em3	6	98-965	3	3	0	50.00	0.68
Me2- Em7	7	156-925	5	2	0	71.43	0.82
Me2- Em8	8	134-654	6	2	1	75.00	o.84
Me2- Em9	7	58-758	1	6	0	14.29	0.24
Me2- Em10	8	61-741	6	2	2	75.00	0.84
Me3- Em2	10	56-644	5	5	2	50.00	0.70
Me3- Em7	6	244-957	2	4	0	33.33	0.72
Me3- Em9	5	228-834	3	2	1	60.00	0.72
Me3- Em10	6	171-629	6	0	1	100.00	0.91
Me4- Em1	11	72-731	7	4	1	63.64	0.80
Me4- Em2	11	35-787	8	3	1	72.73	0.89
Me4- Em3	6	275-1088	2	4	0	33.33	0.72
Me4- Em4	7	109-893	3	4	0	42.86	0.61
Me4- Em5	7	154-1150	3	4	0	42.86	0.61
Me4- Em6	6	159-681	2	4	0	33.33	0.72
Me4- Em7	9	216-1398	5	4	3	55.56	0.72
Me4- Em8	7	161-1004	3	4	0	42.86	0.61
Me4- Em9	7	73-302	4	3	1	57.14	0.74
Me4- Em10	7	78-716	3	4	0	42.86	0.61
Me5- Em1	8	30-672	5	3	0	62.50	0.74
Me5- Em2	11	61-223	5	6	1	45.86	0.48
Me5- Em3	7	123-730	3	4	1	42.86	0.61
Me5- Em4	8	119-756	3	5	0	37.50	0.55
Me5- Em5	8	82-819	3	5	2	37.50	0.55
Me5- Em6	7	148-607	3	4	1	42.86	0.61
Me5- Em7	8	307-650	4	4	2	50.00	0.69
Me5- Em9	8	116-573	4	4	1	50.00	0.66
Total	292		156	136	44	1935.93	
Average	7.9		4.2	3.7	1.19	52.3	0.67

∗PIC: Polymorphism information contents

Table (6): The SRAP genetic similarity (G.S) for the three testers and eight *Zea mays* inbred lines.

	Giza1	S.C.10	T.W.C. 310	S1.19	S1.45	S1.46	S1.50	S1.51	S1.59	S1.61
S.C.10	0.68									
T.W.C. 310	0.68	0.79								
S1.19	0.63	0.63	0.52							
S1.45	0.26	0.58	0.58	0.53						
S1.46	0.37	0.47	0.37	0.74	0.58					
S1.50	0.32	0.53	0.42	0.58	0.84	0.63				
S1.51	0.47	0.47	0.58	0.52	0.26	0.58	0.32			
S1.59	0.32	0.32	0.11	0.47	0.42	0.63	0.58	0.53		
S1.61	0.37	0.37	0.58	0.32	0.47	0.26	0.53	0.58	0.42	
S1.64	0.53	0.63	0.53	0.37	0.53	0.42	0.58	0.42	0.47	0.42

Table (7): Genetic similarity (G.S) of combined SSRs and SRAP results for the three testers and eight *Zea mays* inbred lines.

	Giza 1	S.C.10	T.W.C. 310	S1.19	S1.45	S1.46	S1.50	S1.51	S1.59	S1.61
S.C.10	0.69									
T.W.C. 310	0.70	0.78								
S1.19	0.63	0.65	0.60							
S1.45	0.55	0.57	0.61	0.64						
S1.46	0.54	0.52	0.55	0.67	0.61					
S1.50	0.51	0.59	0.61	0.57	0.62	0.66				
S1.51	0.52	0.61	0.59	0.59	0.57	0.65	0.59			
S1.59	0.50	0.56	0.53	0.57	0.57	0.57	0.63	0.65		
S1.61	0.50	0.56	0.57	0.46	0.55	0.56	0.63	0.70	0.67	
S1.64	0.54	0.58	0.57	0.53	0.51	0.56	0.58	0.60	0.66	0.66

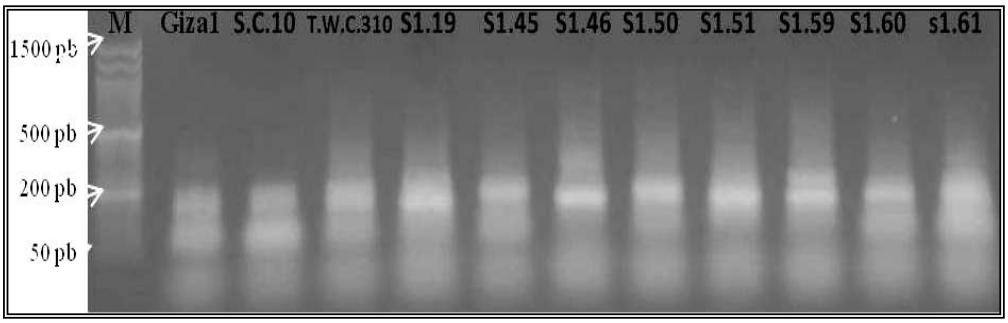

Fig. (1): An example of DNA polymorphism of the eleven *Zea mays* genotypes using (Phi022) SSRs marker.

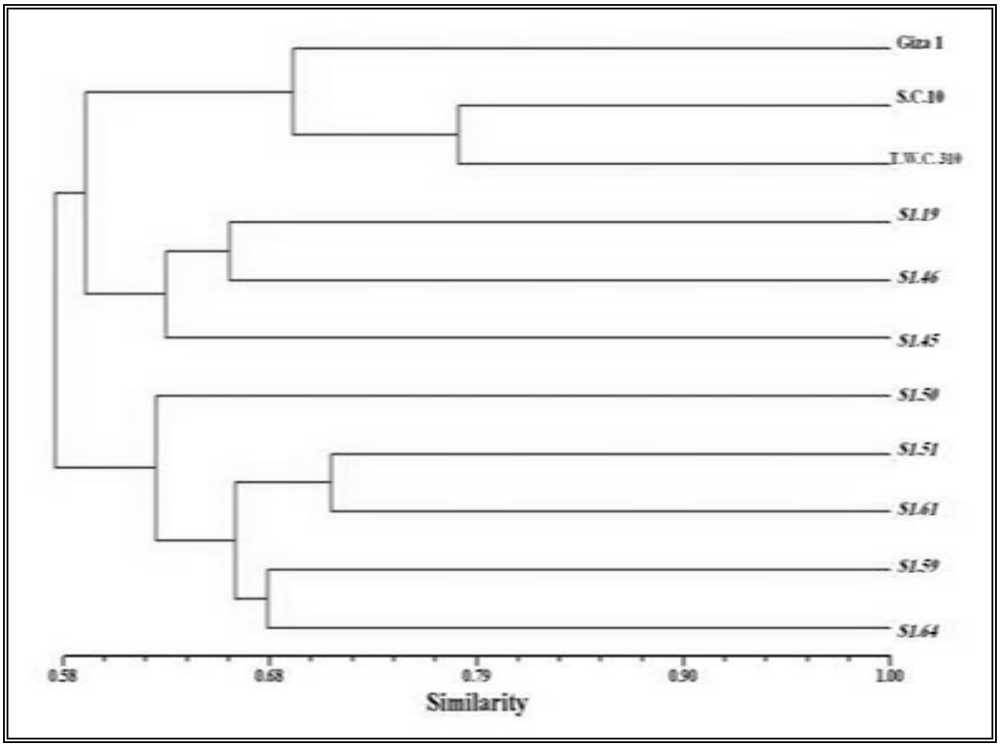

Fig. (2): The SSRs dendrogram for the three testers and eight *Zea mays* inbred lines.

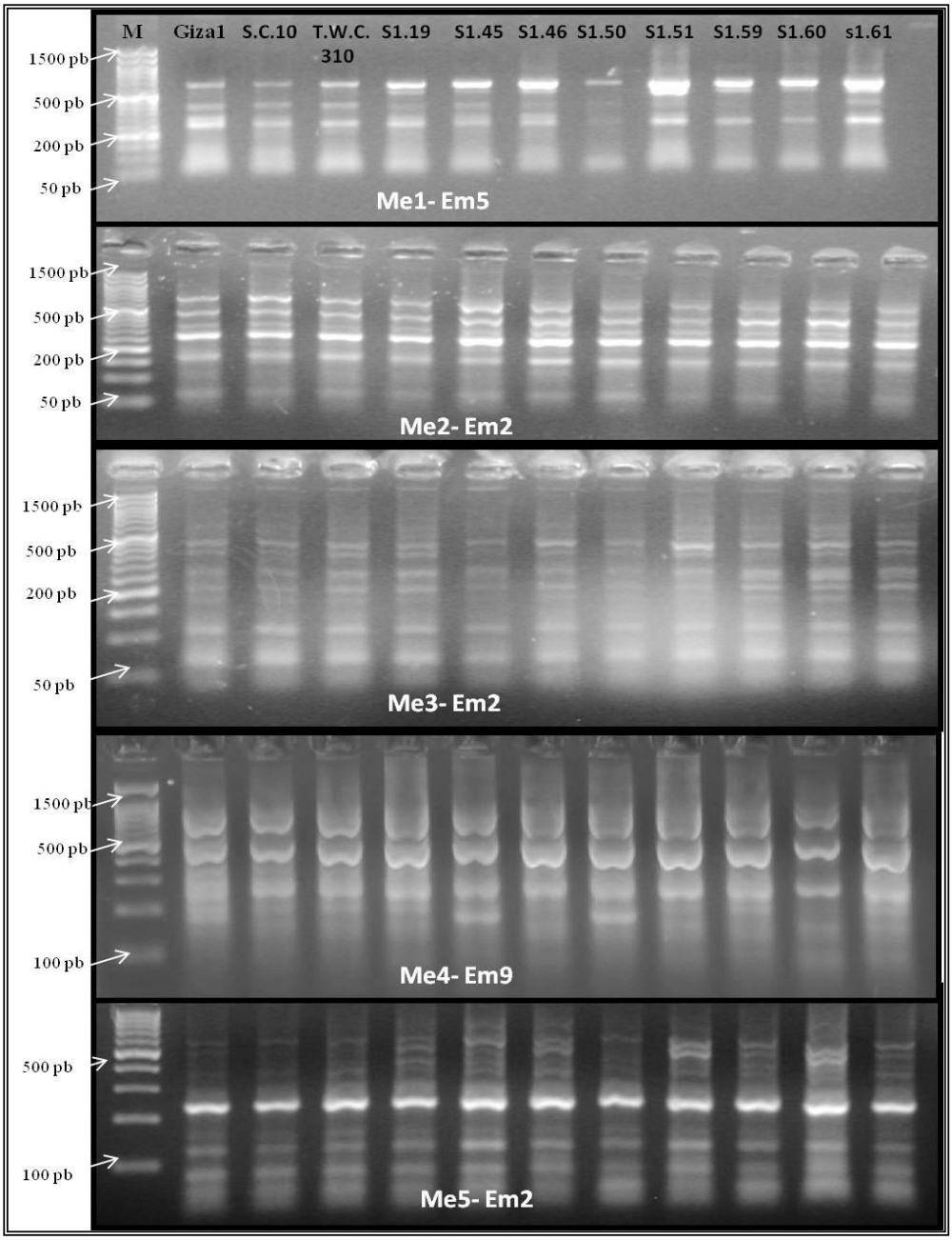

Fig. (3): An example of DNA polymorphism of the eleven *Zea mays* genotypes using SRAP markers.

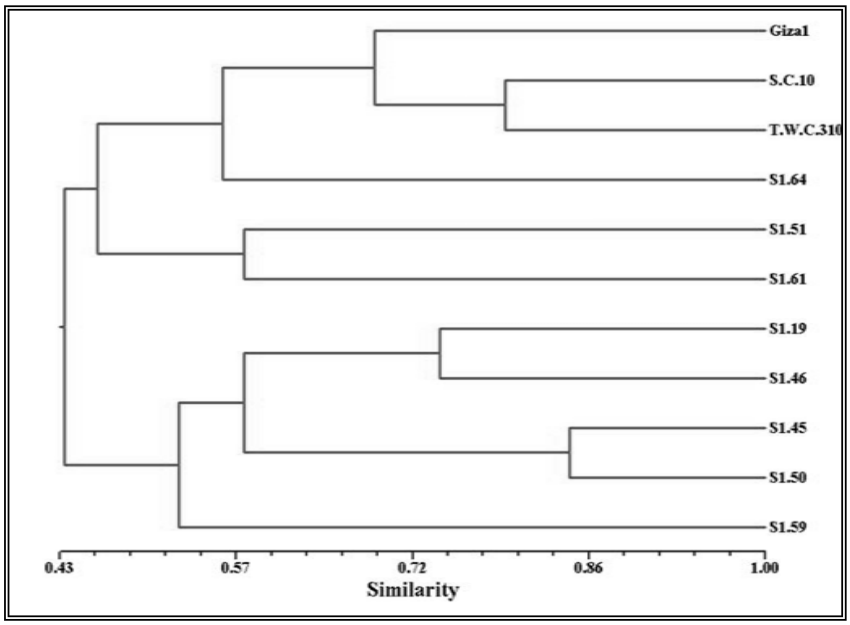

Fig. (4): The SRAP dendrogram for the three testers and eight *Zea mays* inbred lines.

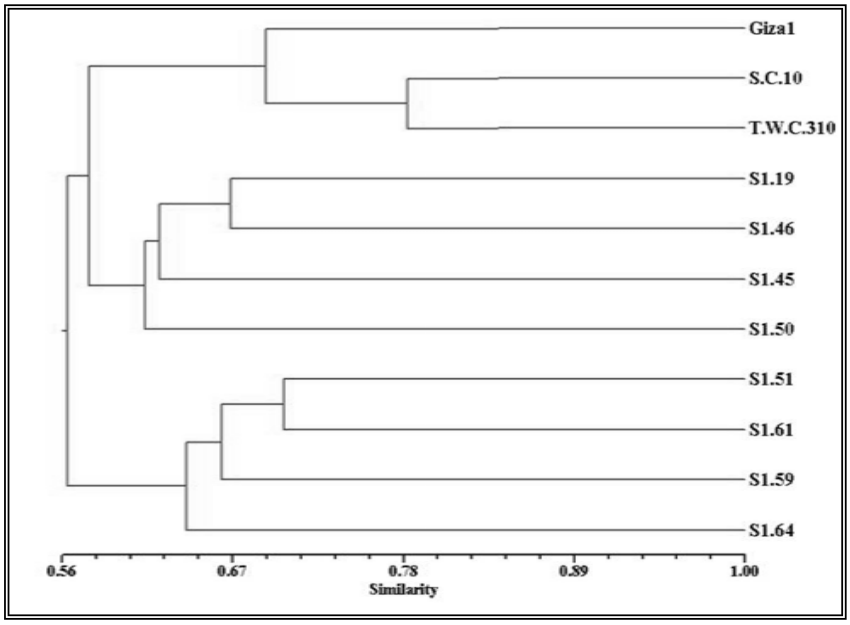

Fig. (5): Dendrogram of combined SSRs and SRAP results for the three testers and eight *Zea mays* inbred lines.