U. of K. J. Agric. Sci. 27, 1-16 , 2019

Estimation of the Genetic Diversity among Twenty Guar (*Cyamopsis tetragonoloba* L. Taub) Genotypes Using Molecular Markers[1]

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(Received 30 /01/2020, Accepted 12 /09/2020, Published on line October 2020)

Abstract: The aim of this study was to determine the genetic diversity and relationships among twenty newly developed genotypes of *Cyamopsis tetragonoloba* using Randomly Amplified Polymorphic DNA (RAPD) molecular technique. Data obtained from application of ten molecular markers were subjected to the procedure of clusters analysis. Random Amplified Polymorphic DNA (RAPD) molecular markers indicated the presence of a wide genetic diversity among the studied twenty genotypes of guar. The constructed dendrogram, based on Jaccard's similarity coefficients, differentiated the genotypes into seven main clusters. The closest genotypes were genotypes Gm34 and L53 with (98 % similarity), while the most diverse ones were genotype L14 and genotype Gm16 with 29% genetic similarity. It was concluded that use of RAPD markers can be used for efficient estimation of genetic diversity in guar. Hence, the current investigation can contribute to enhancement of molecular breeding approach for the development of improved guar genotypes.

Key words: Guar**;** genotypes; genetic diversity; RAPD.

[1] Extracted from a Ph.D. thesis by the first author submitted to University of Khartoum, Faculty of Agriculture, Shambat, Sudan.

INTRODUCTION

Guar (*Cyamopsis tetragonoloba* L. Taub) belongs to the family Leguminosae (Fabaceae) (2n = 14), is a drought tolerant annual legume crop. It is an important legume which cultivation is mainly concentrated in marginal and sub marginal soils receiving low rainfall. Guar seed is highly valued in numerous industries because of its galactomannan rich endosperm. Guar galactomannan is also known as guar gum and is used as a viscosity enhancer for both food and non food purposes (Sabahelkheir *et al.* 2012).

Despite the great significance of this species, only few reports exist for the development of genomic resources in guar (Dhugga *et al*. 2004). Therefore, attempts to analyze possible untapped genetic diversity become extremely essential for breeding and crop improvement. Molecular markers have been applied widely in genetic analyses and breeding studies, as well as investigations of genetic diversity and relationships between cultivated species. These methods have several advantages over morphological traits, including high polymorphism rate and independent effects related to environmental conditions and the physiological stage of the plants. Several DNA marker systems are used in assessing genetic diversity of plants. Among all the molecular markers, random markers like RAPDs and ISSRs are most widely used because they are inexpensive, quick, simple and do not require sequence information (Zietkiewicz *et al*. 1994). RAPD being a multi locus marker with the simplest and fastest detection technology, has been used for diversity analysis in several tree species (Vaishali *et al*. 2008) and many crop species (Agarwal *et al*. 2008).

RAPD is a dominant PCR - based DNA marker technique, which employs single decamer primer of arbitrarily sequence for differential amplification of genomic DNA (Williams *et al*. 1990). The Polymorphism in the RAPD profile is produced by rearrangement of the deletion at or between oligonucleotide primer binding sites in the genome, which causes absence or presence of a band in gel electrophoresis (Rafalski and Tingey 1993). This method is quick, simple and requires less amount of DNA per assay. Being based on random primer, it does not require prior knowledge of sequence information of its design. These markers systems have been used more

frequently for genetic diversity studies, variety identification and for understanding genetic relationships in crop species (Rafalski and Tingey 1993). However, information regarding the application of molecular markers techniques for assessing genetic diversity in guar is meager. In an investigation of genetic differences among 31 guar genotypes, Shabarish Rai and Dharmatti (2013), indicated that they could be grouped into three clusters. Sultan *et al*. (2013), in study of diversity among 30 guar accessions, indicated that they could be classified into three main groups and 9 sub-groups or clusters, with similarity coefficients ranging between 0.49 and 0.93.

The objective of this study was to estimate the genetic diversity among 20 newly developed guar genotypes using RAPD molecular markers.

MATERIALS AND METHODS

Plant Materials

Seeds of twenty newly developed genotypes of *Cyamopsis tetragonoloba* L. Taub (Gm2, Gm4, Gm5, Gm6, Gm7, Gm8, Gm9, Gm16, Gm17, Gm18, Gm19, Gm21, Gm22, Gm23, Gm24, Gm29, Gm31, Gm34, L14 and L53) were kindly provided by Dr. Abdel wahab H. Abdalla, Department of Agronomy, Faculty of Agriculture, University of Khartoum. The studied genotypes were derived by classical method of breeding for the improvement of gum quality.

DNA extraction and RAPD-PCR

Leaf samples from two weeks old guar seedlings were used for DNA extraction. DNA extraction protocol was followed for DNA extraction from each genotype, using modified CTAB (cetyl trimethyl ammonium bromide) protocol, which is a modified method described by Mace *et al*. (2004). The amplification reaction contained 5× FIREPol PCR Master H_2O mix (ready to load), 5x reaction buffer (0.4 M Tris-HCl, 0.1 M $(NH_4)_2$ SO_4, 0.1% w/v Tween-20), 12.5 mM$MgCl_2$, 1 mM each of dNTPs, 50 ng of each primer, 1 U Taq polymerase and 20 ng template DNA. Ten Randomly Amplified

Polymorphic DNA (RAPD) primers were used. They were purchased from Gene Link, Inc., and Operon Tech, Inc., USA. The code numbers and sequences of these oligo-primers are depicted in Table 1. The PCR program for the amplification of the primers using a thermal cycler (Techne/Flexigene) included: Initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 1 min, annealing at 35°C for 30s, extension at 72°C for 2 min and final extension step at 72°C for 5 min. PCR products were separated using 1.5 % agarose gel in 1x Tris Borate EDTA (TBE) buffer at 75 V constant voltages for 70 min. Gels were stained with ethidium bromide, under UV light chamber and observed using the Model TM-10E, UVi tec. product. Then the images were captured using a digital camera. The sizes of bands were estimated using 1.5 Kb ladder molecular size markers.

Table (1). Names and sequences of RAPD primers used in the study.

No	RAPD Primer Name	Sequences
1	GLC-15	5' GACGGATCAG 3'
2	OPC-09	5' CTCACCGTCC 3'
3	OPY-01	5' GTGGCATCTC 3'
4	OPL-16	5'AGGTTGCAGG 3'
5	OPK-16	5 'GAGCGTCGAA 3'
6	OPK-17	5 'CCCAGCTGTG 3'
7	OPR-10	5 'CCATTCCCCA 3'
8	OPL-18	5 'ACCACCCACC 3'
9	OPY-14	5' GGTCGATCTG 3'
10	OPY-17	5' GACGTGGTGA 3'

Data analysis

Each DNA fragment obtained by each marker was scored as present (1) or absent (0) and treated as an independent character. Similarity between the investigated guar genotypes was estimated on the basis of the recorded scores. Data were then used to create a matrix to analyze genetic relationship using the PAST software package. Then dendrogram was constructed based

on Jacaard's similarity coefficients, using the unweighted Pair Grouped Method of Analysis as applied by Vendrame *et al.* (1999).

RESULTS AND DISCUSSION

The ten primers used in the present study successfully amplified DNA fragments from guar DNA samples (Plate 1 a-j). The similarity matrix was obtained using Jaccard′s genetic similarity coefficient (Table 2). The similarity matrix was then used to construct a dendrogram (Fig. 1). Cluster analysis and Jaccard′s similarity coefficients indicated that the twenty genotypes could be differentiated into seven main clusters, based on 70 % similarity coefficient. Cluster one included only one genotype L14. Cluster two contained two sub-clusters, of which the first sub-cluster included Gm7, Gm8 and Gm29 and the second one included one genotype Gm23. Cluster three contained two sub-clusters. The first sub-cluster contained two sub sub-clusters, of which the first included Gm18 and Gm19, the second included genotype Gm22, Gm34 and L53. The second sub-cluster included one genotype Gm21. Cluster four included only one genotype Gm16. Cluster five contained two sub-clusters, of which the first sub-cluster included genotype Gm9 and Gm31 and the second sub-cluster included Gm24. Cluster six contained two sub-clusters, of which the first sub-cluster included Gm17 and Gm2 and the second sub-cluster included genotype Gm4. Cluster seven contained two sub-clusters, the first one included genotype Gm6 and the second one included Gm5 (Fig. 1).

Regarding similarity coefficient, the closest genotypes were Gm34 and L53 (with 98 % similarity), followed by Gm8 and Gm29 with (92 % similarity) then by Gm34 and Gm22 as well as genotypes L53 and Gm22 (with 88% similarity between each pair). However, the most diverse ones were L14 and Gm16, with 29% genetic similarity. The present findings indicate wide variation between genotypes. Previous investigations reported genetic differences among 31guar genotypes which were grouped into 3 clusters (Shabarish Rai and Dharmatti 2013). Sultan *et al*. (2013), in study of diversity among 30 guar accessions, indicated that they could be classified

into three main groups and 9 sub-groups or clusters, with similarity coefficients ranging between 0.49 and 0.93.

Based on the results obtained in the present study, it can be concluded that molecular evaluation and assessment of genetic variation of guar genotypes through RAPD markers is quite efficient to differentiate genetically the evaluated 20 genotypes of guar. In crop improvement, the genetic advance from selection is greatly dependent on the magnitude of the variation present in the plant material under selection. Crossing of genotypes which exhibit a high degree of similarity would result in a low extent of variability and consequently a low genetic advance from selection. On the other hand, crossing of genotypes with a low degree of similarity would generate a wide extent of variability, consequently a high genetic advance would be expected from selection. Hence, the current investigation can contribute to enhancement of molecular breeding approach for the development of improved guar genotypes.

Table (2): Genetic similarity matrix based on RAPD among 20 guar genotypes

Genotypes	gggGm2	Gm4	Gm5	Gm6	Gm7	Gm8	Gm9	L14	Gm16	Gm17	Gm18	Gm19	Gm21	Gm22	Gm23	Gm24	Gm29	Gm31	Gm34	L53
Gm2	1.00																			
Gm4	0.68	1.00																		
Gm5	0.51	0.59	1.00																	
Gm6	0.68	0.51	0.63	1.00																
Gm7	0.43	0.28	0.39	0.59	1.00															
Gm8	0.54	0.40	0.40	0.58	0.86	1.00														
Gm9	0.52	0.37	0.45	0.68	0.64	0.67	1.00													
L14	0.47	0.57	0.57	0.49	0.51	0.60	0.48	1.00												
Gm16	0.68	0.47	0.40	0.60	0.54	0.52	0.48	0.29	1.00											
Gm17	0.83	0.61	0.42	0.56	0.43	0.54	0.56	0.43	0.61	1.00										
Gm18	0.62	0.48	0.51	0.61	0.70	0.78	0.53	0.59	0.57	0.55	1.00									
Gm19	0.59	0.45	0.55	0.64	0.71	0.65	0.54	0.47	0.68	0.52	0.85	1.00								
Gm21	0.71	0.54	0.43	0.54	0.51	0.61	0.48	0.37	0.72	0.67	0.72	0.71	1.00							
Gm22	0.72	0.55	0.53	0.67	0.63	0.74	0.58	0.53	0.68	0.66	0.84	0.78	0.73	1.00						
Gm23	0.49	0.32	0.46	0.60	0.78	0.73	0.70	0.40	0.62	0.54	0.61	0.66	0.54	0.71	1.00					
Gm24	0.69	0.52	0.36	0.56	0.53	0.67	0.68	0.49	0.51	0.69	0.56	0.45	0.59	0.57	0.56	1.00				
Gm29	0.57	0.43	0.41	0.59	0.79	0.92	0.69	0.54	0.55	0.57	0.79	0.67	0.64	0.78	0.77	0.71	1.00			
Gm31	0.62	0.45	0.44	0.63	0.53	0.60	0.76	0.45	0.52	0.71	0.52	0.51	0.50	0.67	0.69	0.70	0.64	1.00		
Gm34	0.68	0.52	0.54	0.65	0.62	0.69	0.55	0.50	0.69	0.61	0.82	0.80	0.74	0.88	0.65	0.52	0.72	0.60	1.00	
L53	0.68	0.53	0.52	0.65	0.62	0.69	0.53	0.50	0.69	0.63	0.82	0.80	0.74	0.88	0.63	0.50	0.72	0.60	0.98	1.00

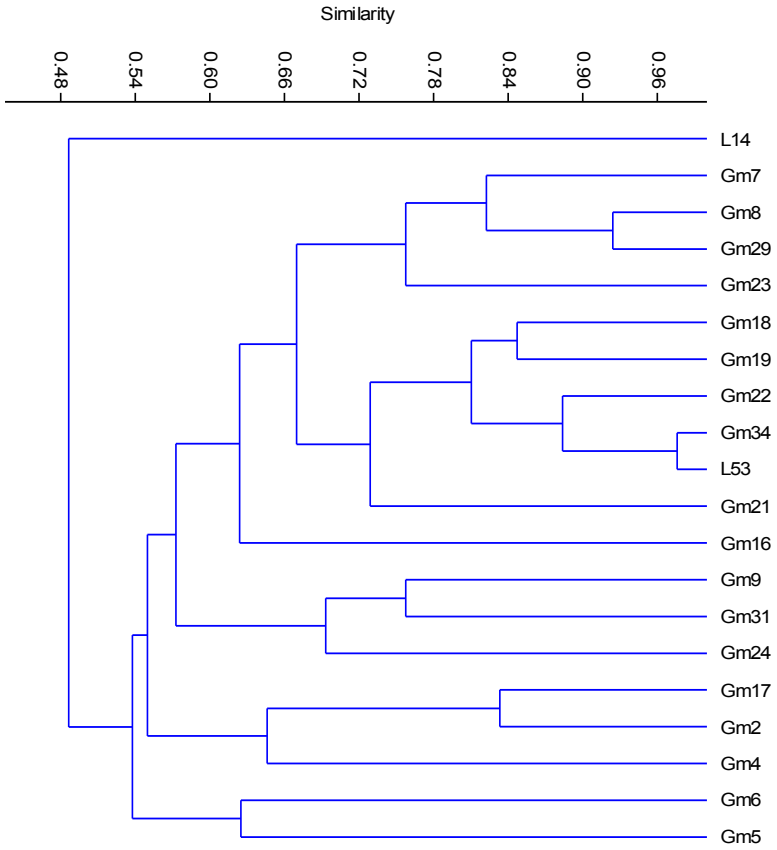
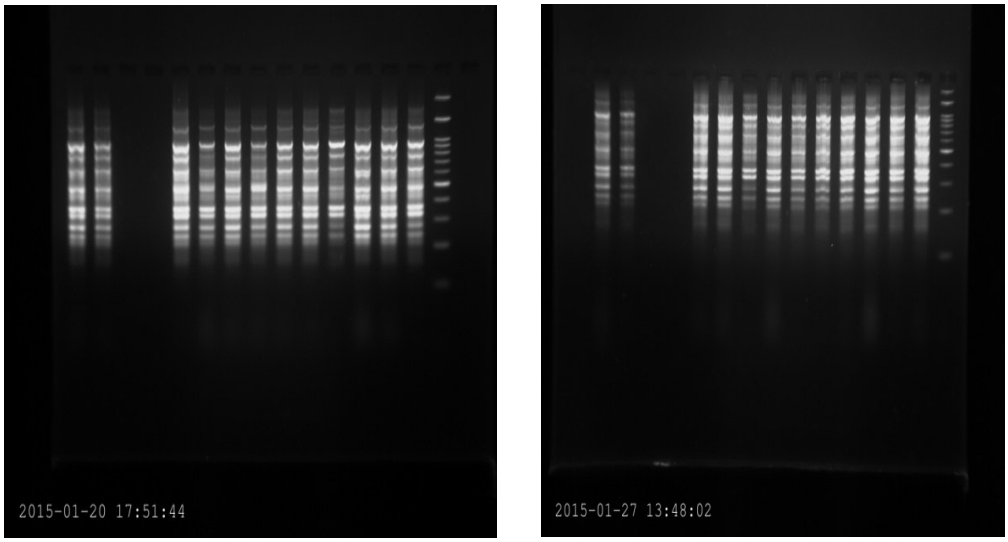
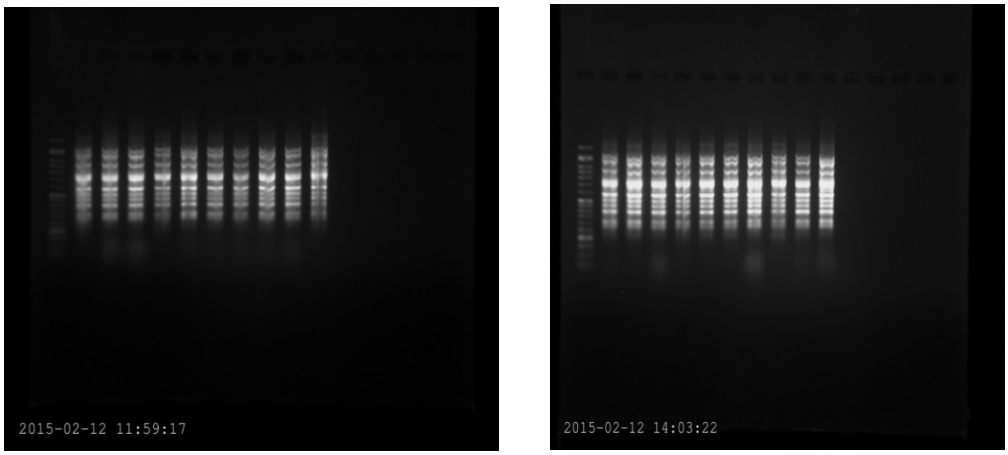


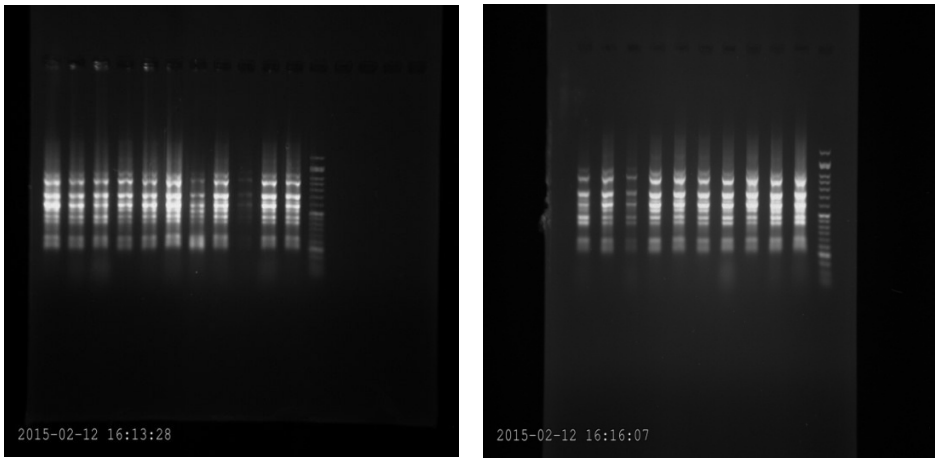
Fig. 1. Dendrogram of twenty guar genotypes showing genetic distances based on RAPD data by using Jaccard's Similarity Coefficient cluster analysis



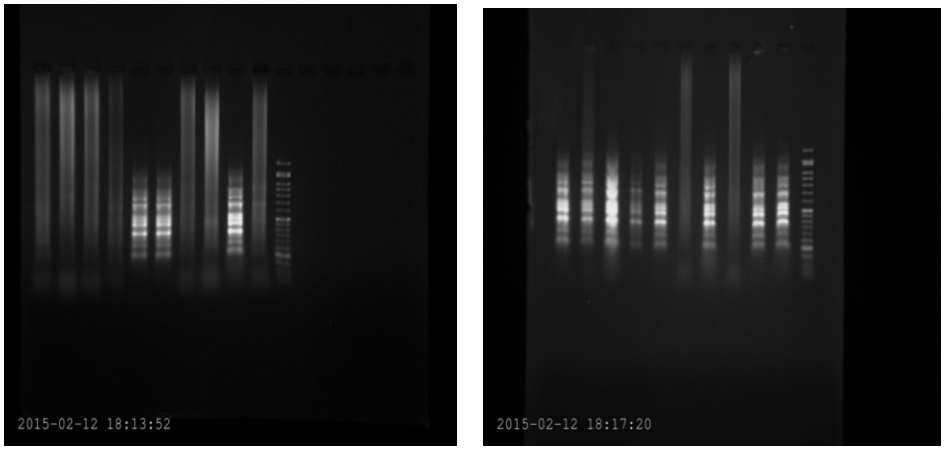
(a). Primer GLC-15



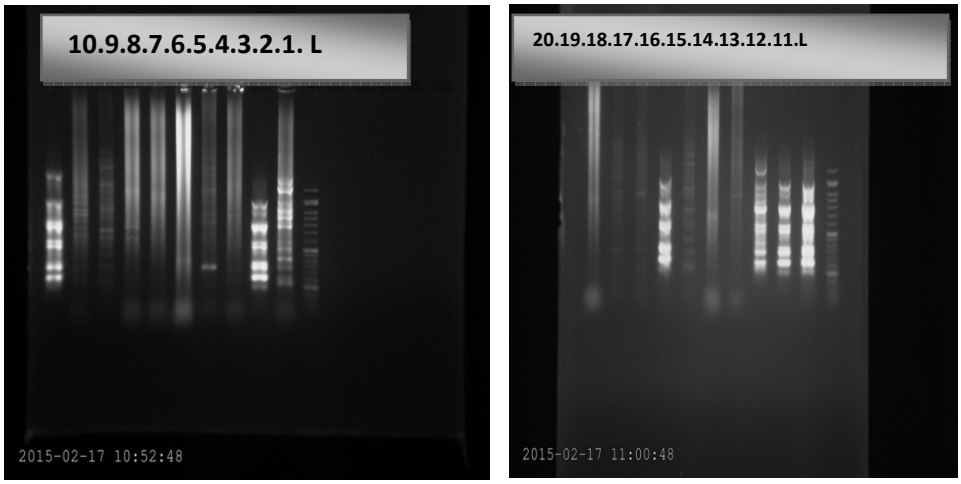
(b). Primer OPC-09



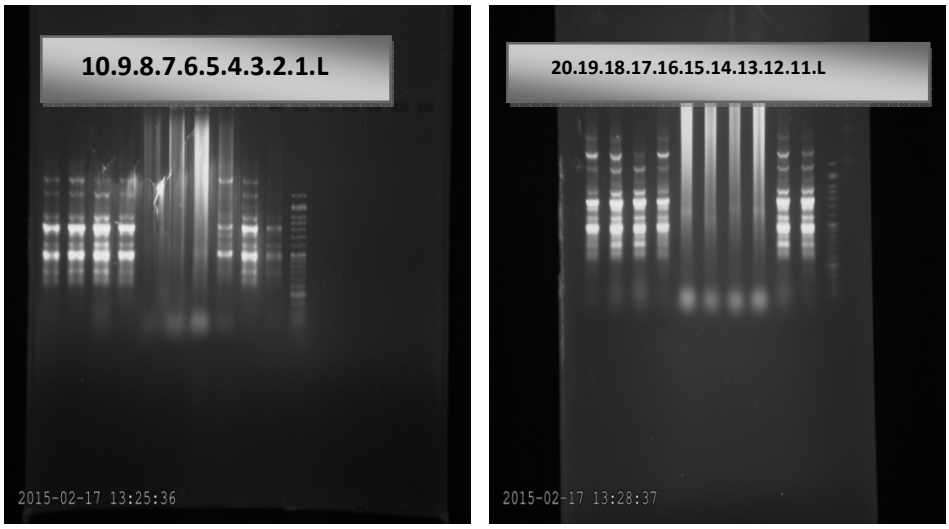
(c). Primer OPY-01



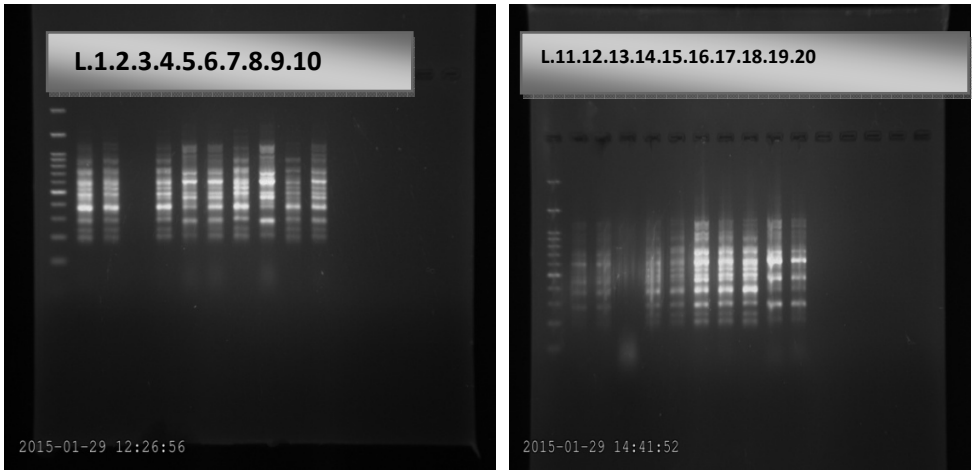
(d). Primer OPL-16



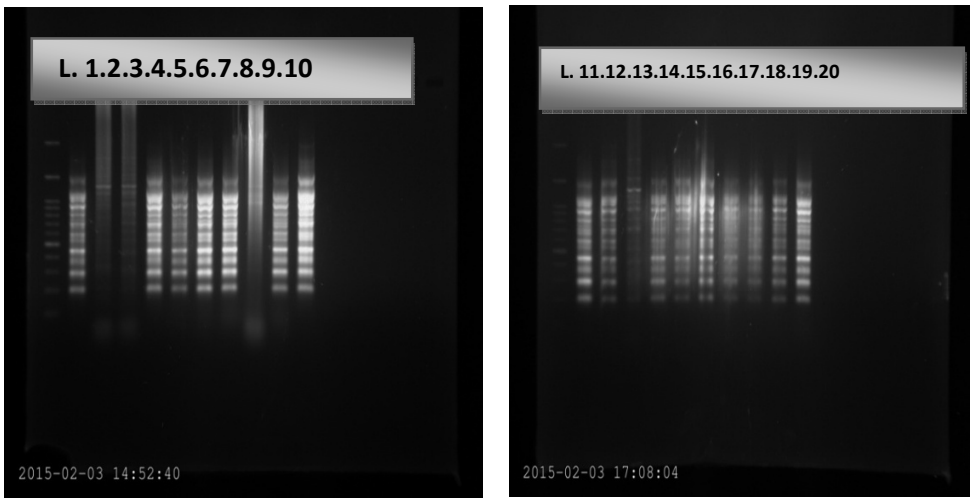
(e). Primer OPK-16



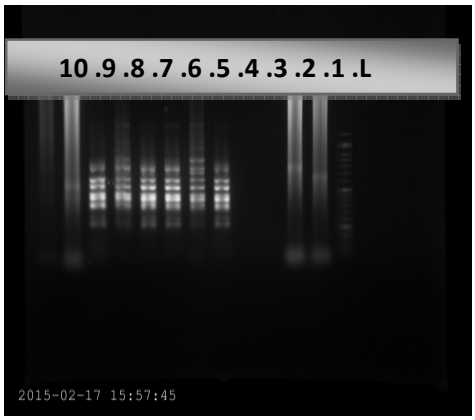
(f). Primer OPK-17

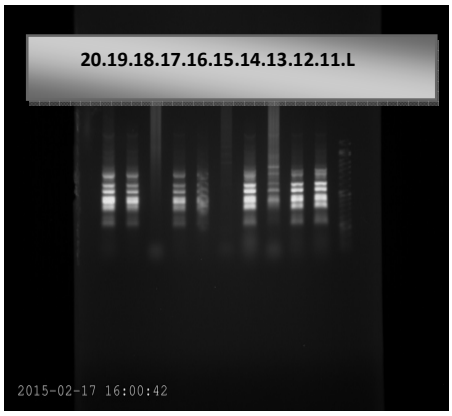


(g). Primer OPR-10

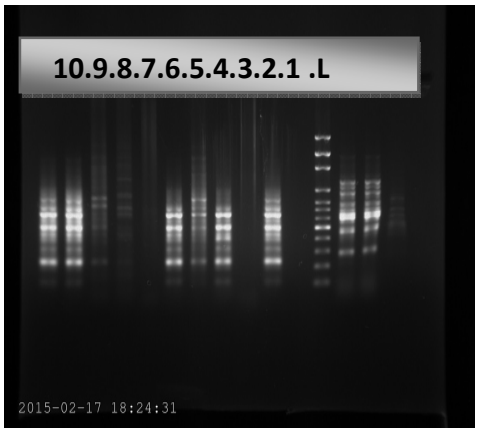


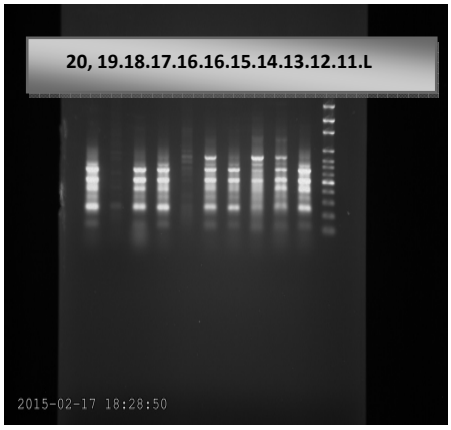
(h). Primer OPL-18





(i). Primer OPY-14





(j). Primer OPY-17

Plate 1. Banding profile (a – j) of twenty guar genotypes genomic DNA amplified by ten RAPD primers (L: ladder 100-1500 Kb, 1= Gm2, 2= Gm4, 3= Gm5, 4= Gm6, 5= Gm7, 6= Gm8, 7= Gm9, 8= L14, 9= Gm16, 10= Gm17, 11= Gm18, 12= Gm19, 13= Gm21, 14= Gm22, 15= Gm23, 16= Gm24, 17= Gm29, 18= Gm31, 19= Gm34, 20= L53).

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تقدير التباين الجيني بين عشرين طراز جديد من القوار (*Cyamopsis tetragonoloba* L. Taub) بإستخدام تقانة الواسمات الجزيئية[2]

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المستخلص : الهدف من هذه الدراسة هو تحديد التباين الوراثي والعلاقات بين عشرين طراز جديد من القوار بإستخدام تقنية التضخيم العشوائي متعدد الأشكال للحمض النووي . أخضعت البيانات التي تم الحصول عليها من تطبيق عشره من الواسمات الجزيئية العشوائية لإجراء تحليل المجموعات (التحليل العنقودي). أشار إستخدام الواسمات الجزيئية العشوائية إلى وجود مدي واسع من التباين الوراثي بين العشرين طراز من القوار. فصل المخطط المبني على أساس معامل التشابه لجاكارد الطرز الى سبع مجموعات. كان أكثر الطرز تشابهاً هى Gm34 وL53 (بمعامل 98%)، بينما كان أبعد طرازين هما L14 وGm16 (بمعامل 29%). خلص البحث إلى أنه بالامكان إستخدام التضخم العشوائي متعدد الأشكال للحمض النووي لتقدير بفعالية التنوع الجيني للقوار. هذه الدراسة يمكن ان تسهم في استخدام التربية الجزيئية لتحسين محصول القوار.

[2] مستلة من اطروحة الدكتوراه للمؤلف الاول