J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 7(10): 263 - 267 , 2016

Assessment of Genetic Diversity Among Egyptian Sorghum Landraces for Grain Yield Variability Using ISSR Markers Analysis

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ABSTRACT

Genetic diversity is an important tool for providing desirable parents during plant breeding programs. Landraces are good source for genetic variability. Thus, this study was performed during two successive seasons to determine the usefulness and genetic diversity among four sorghum (*Sorghum bicolor* L. Moench) landraces they were collected from three different regions in the New Valley governorate of Egypt, in addition to Giza 15 as a control. These genotypes were studied for grain yield performance and inter simple sequence repeat (ISSR) molecular marker analysis. The results showed that all the genotypes were significantly different for grain yield, whereas SL2 landrace produced the highest grain yield of 121.13 g/plant, while the lowest value of 77.13 g was produced by SL3 landrace. In addition, Genetic variability was estimated using ISSR markers where highly polymorphism of 87.8% was observed and moderate similarity relationships were detected. The highest similarity of 0.69 was fond between SL4 and SL2 landraces, but Giza 15 and SL1 showed the lowest similarity of 0.48. Interestingly, UBC846 primer was able to produce negative specific unique band for highly grain yield which would be used as highly grain yield marker. On the other hand, dendrogram analysis isolated the check cultivar in one cluster, while all landraces were located in one other cluster. Furthermore, both SL4 and SL2 which collected from the same region formed one group and exceeded the control. The diversity that observed among landraces and superiority of some of them in grain yield suggested that landraces could be a beneficial tool for sorghum improvement.
Keywords: Sorghum, landraces, ISSR, genetic diversity, dendrogram.

INTRODUCTION

Sorghum is belongs to *poaceae* family and *sorghum* genus. The most cultivated sorghum is *sorghum bicolor*. Sorghum (*Sorghum bicolor* L. Moench) contains 2n= 2x= 20 chromosomes. It is considered as the fifth important cereal crop in the world and represents the second cereal crop for production in Africa (Gerda and Christopher, 2007). Whereas it is used in several purposes, such in human food, animal feed, in biofuels industry and in other several products (Iqbal *et al*., 2010). It is widely cultivated, especially in arid and semiarid lands and in general it is more tolerant to several abiotic stress, has a wide range of adaptability and can be grown in a wide series of environments, including heat, drought, salinity and flooding (Ejeta and Knoll, 2007; Prasad *et al*., 2008; Ali *et al*., 2009).

Landraces varieties are naturally existed and cultivated for many decades under traditional selection methods by farmers themselves. Although it has high capacity for environmental adaptation (Zeven, 1998), it is considered good source for genetic variability that may harboring many genes for biotic and abiotic stress tolerance (Hegde and Mishra, 2009; Karmakar *et al*., 2012; Shimelis and Laing, 2012). Therefore, collection and evaluation of such genotypes is required to obtain superior parents that could be used in breeding programs. Assessment of genetic diversity level among landraces varieties is required to help the breeders in selecting desirable genotypes. The extent of diversity could be measured by many tools among them morphological and molecular markers (Podlich *et al*., 2004). Morphological approach is often limited because of environmental influence (Muthusamy *et al*., 2008). Therefore, molecular markers would be the efficient tools in the discrimination among different genotypes. Among several PCR based molecular markers Inter Simple Sequence Repeats or ISSR have been widely used to study genetic diversity (Fernández *et al.*, 2002).

The main aim of this study was to determine the genetic diversity among 4 Egyptian Sorghum (*Sorghum bicolor* L. Moench) landraces comparing with Giza15 as a check cultivar using both grain yield and ISSR molecular marker analysis for evaluation.

MATERIALS AND METHODS

Plant material:

Four Sorghum (*Sorghum bicolor* L. Moench) landraces varieties namely, SL1, SL2, SL3 and SL4 were collected from three different regions in The New Valley governorate of Egypt, and one check cultivar, Giza 15 (provided by Agricultural Research Center, Egyptian ministry of Agriculture) were utilized in this work. The collection of landraces was achieved from, Balat region for LS4 and LS2, mout region for LS3 and Elrashda village region for LS1 landrace.

Field experiment: Seeds were grown at private farm in Balat, The New Valley governorate during the two summer seasons of 2014 and 2015 in a randomized complete blocks design (RCBD) with a three replications. The cultivation date was in the 1^{st} of June for both experiments. The experimental unit contained five ridges 3.5 meter in length and 60 cm between ridges with 20 cm between plants. After two weeks, seedlings were thinned to one seedlings/hill. All farming applications were performed as recommended (Egyptian ministry of Agriculture) For measuring grain yield (g) at harvest time ten plants were randomly taken from each plot (genotype).

Molecular characterization:

DNA extraction: Genomic DNA was extracted from fresh leaves as described by Xin and Chen (2012).

PCR amplification and electrophoresis: Seven Primers of ISSR markers namely UBC 810, UBC 811, UBC 815, UBC 826, UBC 840, UBC 846 and UBC 861 from EZBiolab-USA were used in this study. PCR amplification reactions were performed according to procedures of Ben El Maati *et al*., (2004) then 1.5% agarose gels were used for immigrating of PCR products, followed by ethidium bromide staining for visualization. The identified bands were counted as 1 (present) and 0 (absent). Nei-Li's similarity index (Nei and Li, 1979) was used for calculation of Genetic similarity. A dendrogram was constructed according to similarity matrix data by unweighted pair group method with arithmatic average (UPGMA), The MEGA program software was utilized in achievement of cluster analysis.

Statistical analysis:

Analysis of variance (ANOVA) was made according to Gomez and Gomez (1984) for a RCBD. Mean comparisons were performed using Least Significant Differences (L.S.D) test at 0.05 level of significance.

RESULTS AND DISCUSSION

A. Grain yield evaluation:

The combined analysis of variance for grain yield is presented in Table 1. The results showed the presence of highly significant differences among all genotypes in grain yield. In addition, the mean performance of all the genotypes in Table 2 revealed that SL3 landrace had the lowest mean value of 77.14 g, while the highest mean value of 121.13 g was obtained by SL2 landrace and followed by SL4 (112.47 g). Clearly, SL2 and SL4 surpassed the other genotypes including Giza 15. Many researchers have studied yield of different sorghum landraces and obtained significant differences among tested genotypes (El Naim *et al*., 2012, Abdel-Fatah *et al*., 2013, Ghani *et al*., 2015 and Akatwijuka *et al*., 2016). Thus, our results are in harmony with their findings. Furthermore, these variations that observed in grain yield may be due to alteration in genetic structure of genotypes (Amanullah *et al*., 2007 and El Naim *et al*., 2012). This complex trait would be controlled by a polygenetic system and greatly influenced by environmental conditions.

Table 1: The mean squares for grain yield of four sorghum landraces and one check cultivar over two years.

S.O.V.	D.F.	Grain yield
Year (Y)	1	241.79**
Error a [Y(r)]	4	29.12
Genotype (G)	4	1168.26**
Y x G	4	902.75**
Error b	16	9.01

**** indicate significant at 1% levels of probability.**

Table 2: The mean performances of four Egyptian sorghum landraces and Giza 15 check cultivar for grain yield/plant (g).

Genotypes	Grain yield/plant (g)
Giza 15	86,65
SL1	89,72
SL2	121,13
SL3	77,13
SL4	112,47
L.S.D 5%	3.60

B. ISSR marker Analysis:

In order to determine genetic diversity among evaluated landraces and Giza 15 cultivar, seven ISSR primers were used in this study as presented in Table 3 and Fig. 1. The results in Table 3 showed that seventy four bands were detected at size extended from 222 bp to 1400 bp. Whereas, among the seven tested primers, UBC810 produced the highest number of bands (16 bands), while the lowest band numbers showed by UBC815 primer. On the other hand, 65 bands were polymorphic with an average of 9.29 per primer. Moreover, all the primers showed polymorphism ratio ranged from 28.57% to 100% with an average of 84.24% and the polymorphism was 87.8%. Remarkably, the level of estimated polymorphism was high indicating the presence of significant variability among evaluated landraces. The present results are in consistent with Taher *et al*. (2015). On the other hand, one negative specific unique band for highly grain yield was detected by UBC846 prime at size of 884 bp that only present in low grain yield genotypes but absent in SL2 and SL4 which showed the highest grain yield varieties. These results in are consistent with previous studies by Ammiraju *et al*. (2002) and Khaled and Hamam (2015) who detected several ISSR markers associated with different agronomic traits. These specific markers are useful for selection of higher grain yield genotypes.

Table 3: Polymorphism obtained by seven ISSR primers in four Egyptian sorghum landraces and Giza 15 check cultivar.

Primers	Primer sequence	Range of fragment size bp	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism %
UBC 810	$(GA)_8T$	222-1400	16	0	16	100
UBC 811	$(GA)_8C$	260-1241	12	0	12	100
UBC 815	$(CT)_8G$	255-545	7	5	2	28.57
UBC 826	$(AC)_8C$	325-1163	9	1	8	88.90
UBC 840	$(GA)_8YT$	226-753	12	2	10	83.3
UBC 846	$(CA)_8RT$	338-884	9	1	8	88.90
UBC 861	$(ACC)_6$	420-1353	9	0	9	100
Total		222-1400	74	9	65	
Average			10.57	1.29	9.29	84.24

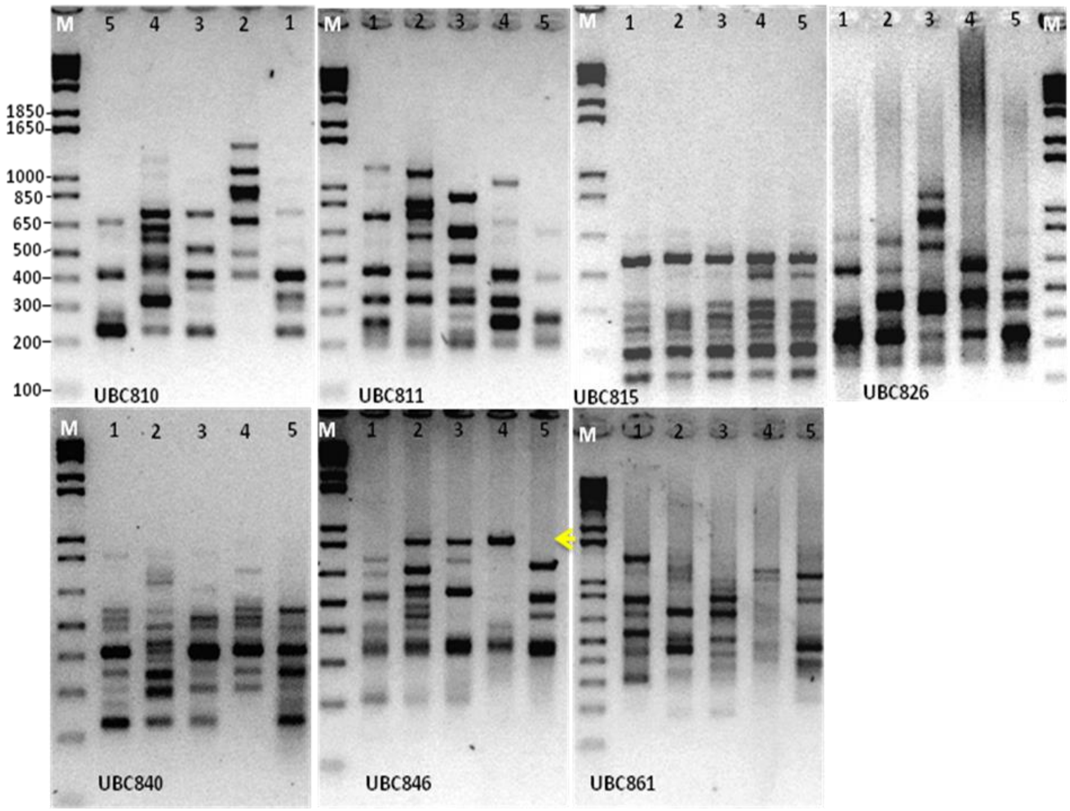

Figure 1, ISSR-PCR amplified fragments produced by seven primers for four Egyptian sorghum landraces and Giza 15 check cultivar (1, SL2; 2, SL1; 3, Giza15; 4, SL3; 5, SL4). M, DNA ladder, yellow arrow shows the negative unique band.

Based on ISSR recorded data, cluster analysis were performed to generate dendrogram using Nei-Li's similarity index. The results in Table 4 showed that the highest similarity of 0.69 was round between SL2 and SL4 landraces, while the lowest similarity of 0.48 was noted between SL1 landrace and Giza 15 cultivar. Furthermore, the UPGMA cluster analysis isolated the four landraces and the check cultivar into two main clusters, the first one included the check Giza 15 cultivar, while the second main cluster contained all the four landraces. Whereas, this second cluster was divided into two sub-clusters, one of them contained only SL1 landrace, while the other one included two groups, the first group consisted of SL3 and the second one gathered both SL4 and SL2 landraces which showed the closest values of grain yield (112.47 and 121.13g/plant, respectively (Table 2). It is clearly that the dendrogram could be able to separate the landraces according to their region. These results were in harmony with many studies by Alhajturki *et al*. (2012), Tadesse and Feyissa (2013), Kimani *et al*. (2014) and Taher *et al*. (2015). Additionally, the close relation which observed between SL2 and SL4 landraces that may be due to these genotypes have been collected from the same region and may be genetically more related (Ghalmi *et al*., 2010). Moreover, the moderate genetic similarity values which noted among all landraces suggested that these landraces may be have more one origin.

Table 4: The similarity index among four Egyptian sorghum landraces and Giza 15 check cultivar based on ISSR

Genotypes	Giza 15	SL1	SL2	SL3
SL1	0.48			
SL2	0.57	0.55		
SL3	0.52	0.51	0.61	
SL4	0.53	0.59	0.69	0.60

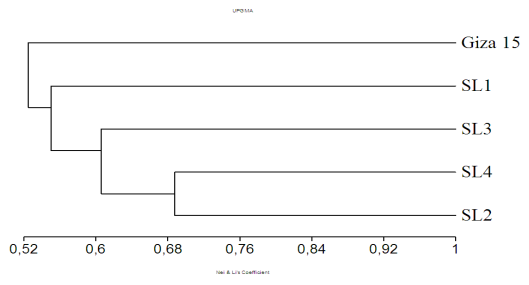

Figure 2: The dendrogram of genetic distances among four Egyptian sorghum landraces and Giza 15 check cultivar using UPGMA cluster analysis of Nei-Li's similarity coefficient based on ISSR markers.

CONCLUSION

In the present study, the extent of genetic diversity among four landraces collected from three different regions of the New Valley governorate of Egypt and the check commercial cultivar Giza15 were evaluated according to grain yield and ISSR marker analysis. The grain yield significantly varied among all genotypes where the SL2 had the highest grain yield. Moreover, ISSR markers successfully detected genetic diversity among these genotypes and they were able to produce negative specific unique band for highly grain yield which could be used as a marker for selection of higher grain yield genotypes. Furthermore, the dendrogram was able to group all the landraces in one cluster while the check cultivar was located in another one. However, the present results suggested that these studied landraces could be beneficial tool for sorghum improvement.

Acknowledgments

We are grateful to The New Valley Agriculture Faculty, Assiut University, Egypt for the financial support of this work.

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تقدير الاختلافات الوراثية بين سلالات برية مصرية من الذرة الرفيعة لتباين محصول الحبوب باستخدام تحليل الواسمات الجزيئية (ISSR)

طلعت بشندي

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التنوع الوراثي هو أداة مهمة لتوفير العديد من الآباء الجيدة لبرامج تربية النبات .حيث تعتبر السلالات البرية من أهم مصادر التباين الوراثي .لذلك هذه الدراسة قد أجريت خلال موسمين متتاليين لتحديد التنوع الوراثي بين أربعة سلالات برية من الذرة الرفيعة و التي تم جمعها من ثلاثة مواقع مختلفة في محافظة الوادي الجديد، مصر، وبالإضافة الي الصنف التجاري جيزة 15للمقارنة ككنترول بواسطة استخدام تقييم أداء محصول الحبوب و تحليل الواسمات الجزيئية .(ISSR)وأظهرت النتائج وجود فروق معنوية بين جميع التراكيب الوراثية في إنتاجية محصول الحبوب .حيث أعطت السلالة SL2أعلى قيمة لمحصول الحبوب 121.13)جم/للنبات (في حين أعطت السلالة SL3أقل قيمة 77.13)جم/للنبات .(أيضا استخدمت الواسمات الجزيئية ((ISSRلدراسة التنوع الوراثي بين هذه السلالات حيث لوحظ ارتفاع نسبة تعدد الأشكال المظهرية 8.87)٪ .(وجود درجة قرابة متوسطة بين جميع التراكيب الوراثية حيث كان أعلى تشابه 0.69بين كل من السلالة SL4و السلالة SL2ولكن أدنى تشابه (0.48)كان بين الصنف جيزة 15و السلالة .SL1أظهر البادئ UBC 846القدرة علي إنتاج حزمة من الـ DNAسالبة الارتباط بالإنتاج العالي للحبوب والتي يمكن أن تستخدم كعلامة للكشف عن السلالات الأكثر إنتاجية لمحصول الحبوب .وعلاوة على ذلك من خلال التحليل العنقودي لوحظ وجود الصنف جيزة 15في مجموعة مفردة بينما جمعت كل السلالات البرية في مجموعة أخري .وقد جمعت كلا من السلالة SL4و السلالة SL2في مجموعة واحدة ربما لأنهما جمعا من نفس المنطقة .التنوع الملاحظ بين السلالات البرية وتفوق البعض في صفة المحصول يشير إلى أن هذه السلالات البرية يمكن أن تكون أداة مفيدة للتحسين الوراثي للذرة الرفيعة.

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