J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 7 (5): 135- 139, 2016

Genetic Diversity of Sugarcane Progenitors from the Scri Germplasm Using Ssr

Khaled, K. A. M.[1]; E. A. M. Amer[2] and A.B.A. El- Taib[3]

1-Genetic Dept., Fac. of Env., Bio Agric. and Food processing, BENI SUEF UNIV. (BSU), Egypt (Khaled.scre@gmail.com)

2-Breeding & Genetic Dept., Sugar Crops Research Inst., ARC, Egypt

3-Agronomy Department, Faculty of Agriculture and Natural Resources, Aswan Univ., Egypt.

ABSTRACT

Twenty-two genotypes (parental strains) of sugarcane were chosen from the germplasm bank of Sugar Crops Research Institute (SCRI), Egypt to study diversity and relationship among sugarcane genotypes based on genetic profiles using 18 SSRs primer, twelve of them were sorghum specific primers. The genetic similarity ranged from 3 to 100 % among the 22 sugarcane genotypes. Most dissimilar of all the genotypes was Phil 8013 with genetic distance of 50 %. The cluster analysis classified the 22 sugarcane genotypes into eight groups. Analysis of different SSRs was useful tool for detection genetic diversity to enhance plant breeding programs. Applying SSRs is important specially in sugarcane that can be a troublesome process as a result of the complexness of sugarcane genome. It is often concluded that determine of genetic similarity based on SSRs could give a lot of accurate data to plant breeder. This information can facilitate the exploitation of sugarcane germplasm on molecular basis Future breeding efforts involving crosses within and among the groups identified during this investigation is also helpful for combining useful genes and alleles in new sugarcane genotypes in addition to maintaining genetic diversity

INTRODUCTION

Sugarcane is very important crop because of its economic and its several by-products participating in the international economy all over the world. It is an important source for sugar production, molasses and fresh juice production (Ming *et al*. 2006). Biotechnology participate as tool to increase agricultural productivity in the context of sustainable agriculture (Tecson 2002). Molecular strategies have good edges for sugarcane breeding programs (Alvi *et al*. 2008; Ahmed and Khaled 2009, Khaled 2010, Khaled *et al* 2011, Khaled *et al* 2015).

More specifically, SSR has shown wonderful potential in helping within the identification of quantitative trait loci (QTLs) (Khaled 2010, Khaled *et al* 2011, Khaled *et al* 2015). SSRs have wide used to study the genetic diversity of Saccharum species and also the genetic relationships among them and related genera (Cordeiro *et al*., 2003; Banumathi *et al*., 2010; Khaled 2010, Khaled *et al* 2011, Ukoskit *et al*., 2012 Khaled *et al* 2015). SSR markers have also been important in sugarcane genetic mapping (Aitken *et al*., 2005; Edme *et al*., 2006; Khaled 2010, Khaled *et al* 2011, Khaled *et al* 2015). A lot of studies revealed that molecular markers were used for characterization of Saccharum germplasm, providing a lot of genetic diversity detection (Aitken *et al*., 2005; Khaled 2010, Khaled *et al* 2011, Khaled *et al* 2015).

Among the commonly used markers, SSR has stood out, gaining significant importance in genetic improvement as a result of several desirable attributes, as well as hyper-variability, usually found within the genome of eukaryotes, high polymorphism and co-dominant inheritance (Zhang *et al*., 2010 Khaled *et al* 2015).

The aim of our investigation was study diversity and relationship among sugarcane genotypes based on genetic profiles using SSRs.

MATERIALS AND METHODS

An experimental was carried out in farming and laboratory of Sugar Crops Research Institute (SCRI) during 2014/2015 and 2015/2016 growing seasons to study genetic diversity among sugarcane germplasm.

Plant genotypes

A pool of 22 genotypes of sugarcane (Table 1) was chosen from the germplasm bank of SCRI, Egypt. They were cultivated in Sugarcane Resources Nursery of the SCRI. Selection of material to be use in this study was based on: (1) commercially released Giza (G) genotypes and (2) genotypes commonly used in crosses by SCRI.

DNA extraction and SSR Loci amplification

DNA isolated from meristems using CTAB method described by Doyle & Doyle (1987) and modified by Khaled and Esh (2008). The concentration of DNA was determined by spectrophotometer (using 260 and 280 nm) and by gel electrophoresis in 1% agarose. Eighteen SSR primers were chosen for analysis of sugarcane genotypes, twelve of them were sorghum specific primers. Primers sequences, names and suitable annealing temperature were conducted in Table 2. Amplification was performed as follows; 94ºC for 1 min (one cycle); 94ºC for 20 sec, 50º-55ºC for 35 secs, 72ºC for 45 sec (35 cycles) and final extension at 72ºC for 45 sec (one cycle). Then hold at 4ºC (infinite). The PCR products were conducted to electrophoresis at 90 V, in 2% agarose gel contain 0.5 µg/ml ethidium bromide for approximately 2 h, using 0.5 × TBE buffer, along with a DNA ladder. The gel was visualized under UV.

Table 1: Names, code, origins and pedigrees of the twenty-two sugarcane genotypes.

	Genotype name	Pedigree			Origins
		Female		Male	
1	CO 842	CO 464	X	CO 617	India
2	CP 44-101	CO 281	X	CP 1165	USA
3	CP 55-30	CP43-64	X	CP44-154	USA
4	CP 57-614	C 147-183	X	CP 53-17	USA
5	CP 67-412	CP 44-155	X	CP 53-16	USA
6	G 84-47	NCO 310	X	?	Local seed fuzz
7	G 95-19	Sp 79-2278	X	Sp 80-1043	Local seed fuzz
8	G 98-28	C 34-33	X	?	Local seed fuzz
9	G 2003-44	CP 55-30	X	85-1697	Local seed fuzz
10	G 2003-47	CP 55-30	X	85-1697	Local seed fuzz
11	G 2003-49	CP 55-30	X	85-1697	Local seed fuzz
12	G 2004-27	CP 55-30	X	ROC 22	Local seed fuzz
13	G 2006-6	82-4510	X	70+3898	Local seed fuzz
14	G 2007-61	SP 71-1406	X	CO 842	Local seed fuzz
15	G 2010-7	BS 380	X	V 4001	Local seed fuzz
16	G 2010-8	BU 980	X	BS 495	Local seed fuzz
17	G 2010-9	PR 1117	X	?	Local seed fuzz
18	G 2010-26	BQ 1635	X	CP 70-1133	Local seed fuzz
19	G.T. 54-9	NCO 310	X	F 37-925	Seed fuzz from Taiwan
20	NCO 310	CO 421	X	CO 312	India
21	Phil 8013	CAC 71-312	X	Phil 642227	Seed cutting from The Philippines
22	SP 71-1406	NA 56-79	X	?	Brazil

Table 2: SSR primer names, their sequences and their required annealing temperature for SSR-PCR analysis.

Primers		Forward sequences	Reverse sequences	Ann. Temp.
1-	Xtxp1	TTGGCTTTTGTGGAGCTG	ACC CAG CAG CAG TAC ACT AC	53
2-	Xtxp4	AATACTAGGTGTCAGGGCTGTG	ATG TAA CCG CAA CAA CCA AG	57
3-	Xtxp6	ATCGGATCCGTCAGATC	TCT AGG GAG GTT GCC AC	50
4-	Xtxp8	ATATGGAAGGAAGAAGCCGG	AAC ACA ACA TGC ACG CAT G	53
5-	Xtxp10	ATACTATCAAGAGGGGAGC	AGT ACT AGC CAC ACG TCA C	50
6-	Xtxp12	AGA TCT GGC GGC AAC G	AGT CAC CCA TCG ATC ATC	50
7-	Xtxp17	CGG ACC AAC GAC GAT TAT C	ACT CGT CTC ACT GCA ATA CTG	50
8-	Xtxp19	CTT TAA TCG GTT CCA GAC	CTT CCA CCT CCG TAC TC	57
9-	Xtxp 61	GAT GCC CAT GCC TTG C	CCC ACT AAA CTA AAG CGG AGA	55
10-	Xtxp 65	CAC GTC GTC ACC AAC CAA	GTT AAA CGA AAG GGA AAT GGC	50
11-	Xtxp 141	TGT ATG GCC TAG CTT ATC T	CAA CAA GCC AAC CTA AA	57
12-	Xtxp 357	CGC AGA AAT ACG ATT G	GCT ATC TGG AGT AAC TGT GT	50
13-	Xgwm 191	AGACTGTTGTTTGCGGGC	TAGCACGACAGTTGTATGCATG	60
14-	Xgwm 271	CAAGATCGTGGAGCCAGC	AGCTGCTAGCTTTTGGGACA	60
15-	Xgwm 639	CTCTCTCCATTCGGTTTTCC	CATGCCCCCCTTTTCTG	55
16-	Xgwm 121	TCCTCTACAAACAAACACAC	CTCGCAACTAGAGGTGTATG	50
17-	Xgwm 129	TCAGTGGGCAGCTACACAG	AAAACTTAGTAGCCGCGT	50
18-	Xgwm 573	AAGAGATAACATGCAAGAAA	TTCAAATATGTGGGAACTAC	50

Xtxp = sorghum specific primer
Xgwm = sugarcane primer

Cluster analysis and Genetic similarity

Similarity among genotypes was estimated according to Dice similarity coefficient and UPGMA-based cluster analysis using TotalLab software package v. 2009 provided by nonlinear Dynamics Co. The banding patterns produced were scored and regenerated to binary values of (1) and (0) for the presence and absence of bands, respectively. The binary matrix was analyzed with TotalLab to determine similarity indices among sugarcane genotypes. Comparisons of SSR profiles resulted in a very similarity matrix to develop a consensus tree.

RESULTS AND DISCUSSION

Genotype diversity based on SSR analysis

One of most important component of crop improvement was assessing variability and identification of available germplasm, while genetic distances between different genotypes could be very useful in producing superior crosses to obtain greater genetic gains (Ceron and Angel 2001), SSR-PCR was used successfully in this case.

The SSR-PCR amplification patterns observed in this investigation resolved various degrees of polymorphisms between the 22 sugarcane genotypes. Eighteen (18) SSR primers were chosen to detect polymorphism in 22 sugarcane genotypes. A total of 83 SSR fragments were produced by the 18 primers with an average of 4.61 bands/primer.

Cluster analysis and polymorphism pattern

About 86.75 % polymorphism was estimated as 72 out of 83 fragments were polymorphic with 18 primers used among the 22 sugarcane genotypes. The

rest of the 7 bands were monomorphic. In the present investigation, the 22 sugarcane genotypes appeared to show variability with the 18 primers used (Table 3).

Although none of the primers was as informative as to differentiate all the genotypes; highly polymorphic profiles were obtained with of the primer Xgwm 639. Therefore, conclusion of the present results revealed that SSRs is used for identification of genetic diversity and also the relationship among the complex species. Jannoo *et al*. (2001) used two specific primers to test 96 sugarcane genotypes and found a high level of heterozygosity.

Table 3: Primers name and their band sizes used for detection the polymorphism among sugarcane genotypes

	Primers	Band size		Primers	Band size
1	Xtxp1	600	9	Xtxp 65	405
		720			450
		900	10	Xtxp 141	400
2	Xtxp4	550			600
		900	11	Xtxp 357	305
3	Xtxp6	400			455
		550	12	Xgwm 191	355
4	Xtxp8	400			500
		630	13	Xgwm 271	400
		800			520
5	Xtxp10	500	14		600
		540		Xgwm 639	300
		600		Xgwm 121	350
6	Xtxp12	400	15		550
7	Xtxp17	410	16	Xgwm 129	420
		550		Xgwm 573	550
8	Xtxp19	400	17		650
		500	18	Xtxp 61	515
					700

Cordeiro *et al*. (2001) applied 21 primer sets to five sugarcane genotypes, among them 17 pairs were polymorphic, but they found low level of polymorphism (0.23). Smi ullah *et al* 2013 used SSR primers to detect polymorphism among 17 sugarcane genotypes. A total of 62 fragments were produced by 30 SSR pairs with 2.14 bands/primer.

Genetic similarity ranged from 3 to 100 % among the 22 sugarcane genotypes (Fig. 1). The highest similarity (100 %) was detected among genotypes G 2003-44, G 2003-47 and G 2003-49. This results in harmony with information about this genotypes which produced from the same hybrid (Table 1). Edme *et al* 2006 detected similarity among genotypes S-2003-US-118 and S-2003-US-312 and reported that it was 90.03%.

Polymorphism level indicates that uniqueness between any two genotypes is possible with applicable SSR primer pair. This supports the use of SSR markers, as a superb tool, for diversity analysis and loci mapping

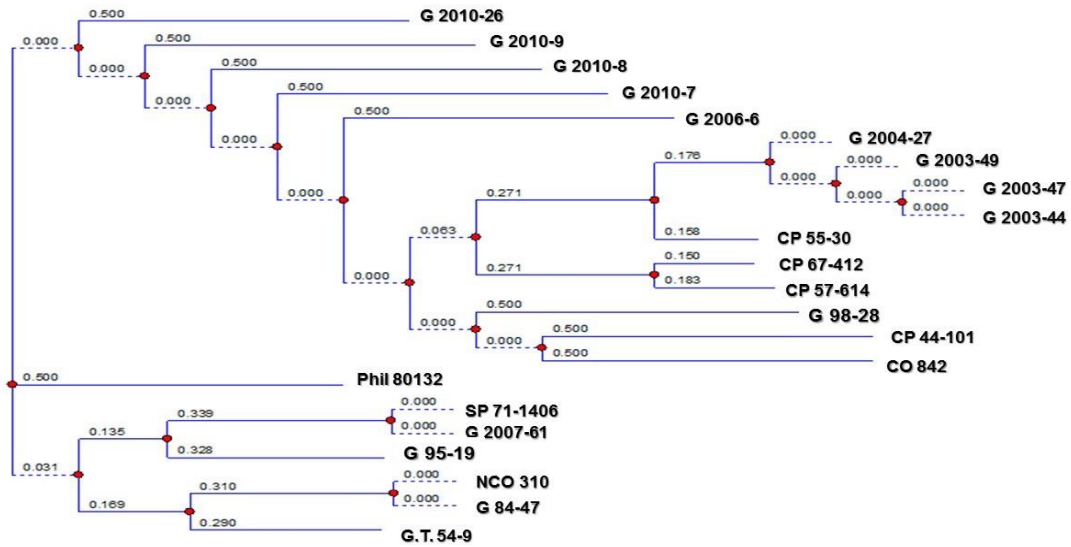

Figure 1: Dendrogram representing the relationships among 22 sugarcane genotypes based on similarity indices derived from SSR analysis

Genetic distances/similarities among genotypes

The genetic distance for SSR data using 22 sugarcane genotypes, was constructed based on Nei (1978) and relationships between genotypes were portrayed graphically in the form of a dendrogram as seen in Figure 1, the value of genetic similarity ranged from 3 to 100 % among the 22 sugarcane genotypes. The most dissimilar of all the genotypes was Phil 8013 which has distance of 50%. SSRs produced greater number of alleles and higher polymorphism level comparing with EST derived SSRs in sugarcane (Pinto *et al*., 2006). Cordeiro *et al*., 2003 reported that sugarcane germplasm was highly diverse. Selvi *et al*. (2003) detected similarity ranged from 0.324 to 0.8335 when tested on 30 or 40 sugarcane cultivars.

Clustering pattern

Cluster analysis based on similarity values classified the sugarcane genotypes into many groups. The first major group divided into three subgroups one of them contain the genotype G 98-28 and the second contain the two genotypes CP 44-101 and CO 842, while the third divided into two subgroups contain the genotypes CP 57-614, CP 67-412, CP 55-30, G 2003-44, G 2003-47, G 2003-49 and G 2004-27.

The second major group also divided into two subgroups, one of them contain the genotypes G.T. 54-9, G 84-47 and NCO 310, while the other contain the genotypes G 95-19, G 2007-61 and SP 71-1406. The reminder six groups each of them contain only one genotype.

CONCLUSIONS

Analysis of SSR fragments was a good tool for detecting genetic diversity to enhance plant breeding programs. Applying SSRs is important especially in sugarcane that may be a difficult method attributable to the complexity of sugarcane genome. Data isn't available on the genetic diversity among and within Saccharrum genotypes. Thus, it is often concluded that determine of genetic similarity based on SSRs might offer additional accurate information to plant breeder. This information can facilitate the exploitation of sugarcane germplasm on molecular basis. Future breeding efforts involving crosses between and among the groups identified during this investigation is also helpful for combining useful genes and alleles in new sugarcane genotypes additionally to maintaining genetic

REFERENCES

Ahmed AZ and Khaled KA 2009. Detection of genetic similarity of sugarcane genotypes. Eelectronic Journal of Gene Conserve 31 (1), 686-697

Aitken KS, Jackson PA and McIntyre CL 2005. A combination of AFLP and SSR markers provides extensive map coverage and identification of homo(eo)logous linkage groups in a sugarcane cultivar. Theor. Appl. Genet. 110: 789-801.

Alvi AK, Iqbal J, Shah AH and Pan YB 2008. DNA-based genetic variation for red rot resistance in sugarcane. Pakistan Journal of Botany 40 (4), 1419-1425

Banumathi, G., V. Krishnasamy, M. Maheswaran, R. Samiyappan, P. Govindaraj, and N. Kumaravadivel. 2010. Genetic diversity analysis of sugarcane (Saccharum sp.) clones using simple sequence repeat markers of sugarcane and rice. Electronic Journal of Plant Breeding 1(4): 517–526.

Ceron A and Angel F 2001. Genetic diversity in sugarcane hybrids bred in Colombia measured using molecular markers. In: Proceedings of the 24th ISSCT Congress, Molecular Biology Workshop, 16-21 September, 2001, Brisbane, Australia, p 626

Cordeiro, G.M., R. Casu, C.L. McIntyre, J.M. Manners, and R.J. Henry. 2001. Microsatellite markers from sugarcane (Saccharum sp.) ESTs cross transferable to Erianthus and sorghum. Plant Science 160(6): 1115–1123.

Cordeiro, GiovanniM, Yong-Bao Pan, and RobertJ Henry. 2003. Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm. Plant Science 165(1): 181–189.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 1987; 19:11–15

Edme SJ, Glynn NG and Comstock JC 2006. Genetic segregation of microsatellite markers in Saccharum officinarum and S. spontaneum. Heredity 97: 366-375.

Jannoo N, Forget L, Dookun A. 2001. Contribution of Microsatellites to Sugarcane Breeding Program in Mauritius. International Society of Sugar Cane Technologists, Proceedings of the XXIV Congress, Brisbane. pp. 637-639.

Khaled A M Khaled. 2010. Molecular characterization and genetic similarity of three sugarcane genotypes. Egypt. J. Genet. Cytol. 39:229-257, July, 2010

Khaled A M Khaled and Esh AMH. 2008. High quality genomic DNA impurities-free from sugar crops and other plant tissue. In: 3rd International Conference IS-2008 "Meeting the Challenges of Sugar Crops & Integrated Industries in Developing Countries", Organizing by International Association of Professionals in Sugar and Integrated Technologies (IAPSIT), 11-14th September, 2008, Al-Arish, Egypt, pp 330-332

Khaled A M Khaled; I.S. El-Demardash, E.A.M. Amer. 2015. Genetic Polymorphism among Some Sugarcane Germplasm Collections as revealed by RAPD and ISSR analyses. Life Science Journal,12(3), 159-167

Khaled, Shereen K. M., F M Abdel-Tawab, Eman M Fahmy and K. A. M. Khaled. 2011. Marker-Assisted Selection Associated with Sugar Content in Sugarcane (Saccharum Spp.). Proceeding of The Third International Conference of Genetic Engineering and its Applications. Sharm EL Sheikh City, South Sinai, Egypt, October 5-8, 2011: 1-13.

Mary, S., Nair, N.V., Chaturvedi, P.K. and Selvi, A. 2006. Analysis of genetic diversity among Saccharum spontaneum L. from four geographical regions of India, using molecular markers. Genet. Resour. Crop Evol. 53, 1221–1231.

Ming, R., P.H. Moore, K.K. Wu, A. D'Hont, Jean C. Glaszman and T.L. Tew. 2006. Sugarcane improvement throuth breeding and biotechnology. Plant Breeding Review, Volume 27.

Nair, N.V., Nair, S., Sreenivasan, T.V. and Mohan, M., 1999. Analysis of genetic diversity and phylogeny in saccharum and related genera using RAPD markers. Genet. Resour. Crop Evol. 46, 73–79.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individual. Genetics 89:583-590.

Pinto, L.R., Oliveira, K.M., Marconi, T., Garcia, A.A.F., Ulian, E.C. and De Souza, A.P. 2006. Characterization of novel sugarcane expressed sequence tag microsatellites and their comparison with genomic SSRs. Plant Breed. 125, 378–384.

Selvi A, Nair NV, Balasundaram N and Mohapatra T. 2003. Evaluation of maize microsatellite markers for genetic diversity analysis and fingerprinting in sugarcane. Genome 46:394-403.

Smi ullah, Farooq AK, Abdullah, Aqeel A, Muhammad AJ, Zafar I, Rameez I and Javed IW. 2012. In vitro Regeneration, detection of Somaclonal Variation and screening for SCMV in Sugarcane (Saccharum spp.) Somaclones. Afr. J. Biotech., 11(48):10841-10850.

Tecson EM. 2002. Crop biotechnology in the Philippines. Ag Biotech Net 4 ABN079, 1-7

Ukoskit K, Thipmongkolcharoen P and Chatwachirawong P. 2012. Novel expressed sequence tag-simple sequence repeats (EST-SSR) markers characterized by new bioinformatic criteria reveal high genetic similarity in sugarcane (Saccharum spp.) breeding lines. Afr. J. Biotechnol. 11: 1337-1363.

Xu, Y.B. and Crouch, J.H. 2008. Marker-assisted selection in plant breeding: from publications to practice. Crop Sci. 48, 391–407.

Zhang, R., Hu, D., Zhang, J., Zuo, X., Jiang, R., Wang, H. and Ng, T.B. 2010. Development and characterization of simple sequence repeat (SSR) markers for the mushroom flammulina velutipes. J. Biosci. Bioeng. 110, 273–275.

التنوع الوراثى لطرز قصب السكر داخل مجموعة الأصول الوراثية المصرية باستخدام تقنية SSR

خالد عدلى محمد خالد[1] , عصام أحمد محمد عامر[2] و اشرف بكرى أحمد الطيب[3]

1- قسم الوراثة - كلية الزراعة البيئية والحيوية والتصنيع الغذائى – جامعة بنى سويف - مصر

2- قسم التربية والوراثة - معهد بحوث المحاصيل السكرية - مركز البحوث الزراعية - مصر

3- قسم المحاصيل - كلية الزراعة والمواد الطبيعية– جامعة أسوان- مصر

فى تجربة لدراسة التنوع الوراثى فى قصب السكر تم اختيار 22 طرز وراثى من مجموعة الأصول الوراثية المصرية لقصب السكر والمحفوظة فى معهد بحوث المحاصيل السكرية حيث تمت دراسة مدى العلاقة بين الطرز المختلفة باستخدام 18 بادئ من بادئات SSR. وقد كانت نسبة التشابه الوراثى تتراوح بين 3-100% وكان الطرز Phil 8013 أكثر الطرز الوراثية أختلافاً عن الأخرين. وقد أسفرت التحاليل الوراثية والإحصائية عن تقسيم الطرز الوراثية من حيث درجة القرابة إلى ثمانية مجموعات حيث تجمعت غالبية الطرز المدروسة فى مجموعتين رئيستين وضمت المجموعات الستة الباقية طرز منفصلة. وقد أثبتت التجربة أن تحليل الاختلافات بين قطع SSR يعتبر أداة فاعلة فى تقدير درجة التشابه والتنوع الوراثى مما يؤدى إلى تحسين استراتيجية برامج التربية. ونظراً لصعوبة التعامل مع قصب السكر والجينوم المعقد له فإن استخدام تقنية SSR يمكن أن يمد مربى النبات بمعلومات دقيقة عن جينوم القصب ونتيجة لهذه الدراسة يمكن تأكيد فاعلية بادئات SSR فى تحديد درجات التشابه والاختلاف الوراثى بين طرز قصب السكر المختلفة ولذلك توصى الدراسة باستخدام يادئات SSR فى دراسات التشابه والتنوع ولاختلاف الوراثى