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Simple Sequence Repeats (SSRs) and Morphological Parametersassociated with Drought Tolerance in Sugarcane *(Saccharum*spp.)

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ABSTRACT

Eight sugarcane genotypes (i.e.G.T.54-9, G84-47, Sp 80-32-80, F.153, Co.997, BOT-41, Co.775, F.161) were selected depending on previous screening of Sugar Crops Research Institute (SCRI) germplasm for detecting morphological parameters and some molecular markers which could assist selection for drought tolerance in sugarcane using Simple Sequence Repeats (SSRs). The eight sugarcane genotypes were screened for drought tolerance depending on five traits (i.e. stalk height, stalk diameter, stalk weight, leaf area and number of stalks /m^2) in two dislocated experiments (sandy culture and open field experiments), where molecular studies were carried out. All studied genotypes were significantly affected by drought in both field and sandy culture experiments. The most affected trait was stalk weight which had the highest reduction percentage (21.55 and 28.93%), followed by No. stalk/m^2 (15.22 and 19.7 %) in sandy culture and field experiments, respectively. The highest reduction percentage was recorded for genotype Co775 with leaf area trait (39.88 %) in field experiment and stalk weight (64.44 %) in sandy culture. The most drought tolerant genotypes were Sp8032-80, Co997 and BOT41, while the most sensitive were Co775, F153 and F161.The performances of studied genotypes revealed the ability of using SSR technique as marker assisted selection for drought tolerance in sugarcane, in this context three positive and negative markers were obtained. Primer SSR15 produced one band with molecular weight 123 bp which could be used as marker for drought sensitivity (negative marker), however, SSR80 produced two bands 24 bp and 35 bp that could be used as negative and positive markers. This data would help the exploitation of sugarcane germplasm on molecular basis, so we highly recommend using SSR as marker assisted selection for drought tolerance in sugarcane.

Keywords: *Sugarcane, SSR, drought, tolerance, Marker assisted selection*

INTRODUCTION

Sugarcane is very important crop because it is an important source for sugar, molasses and juice production (Ming *et al.* 2006). Molecular screening methods have great benefits for many sugarcane breeding programs (Alvi*et al*. 2008; Khaled 2010, Khaled *et al* 2011 and Khaled *et al* 2015). Simple sequence repeat (SSR) has shown to be an excellent potential for assisting and identification of quantitative trait loci (QTLs) (Ming *et al*. 2002; Fratini*et al*. 2007; Khaled *et al*. 2007, Khaled *et al* 2011 and Khaled *et al* 2015). Also, SSR markers have been important in sugarcane genetic mapping studies (Aitken *et al*., 2005; Edme*et al*., 2006; and Khaled *et al* 2016). Several studies have shown that molecular markers have been widely used for the characterization of *Saccharum* complex germplasm, providing a more direct measure of genetic diversity (Aitken *et al*., 2005 and Khaled *et al* 2016). Among the most commonly used markers, microsatellites (SSR) have stood out, gaining considerable importance in genetic improvement due to many desirable attributes, including hyper-variability, often found in the genome of eukaryotic organisms, high levels of polymorphism and co-dominant inheritance (Xu and Crouch, 2008; Zhang *et al*., 2010).

Drought stress alters a majority of physiological processes that ultimately determines yield, (Silva *et al.* 2007). However, drought tolerance is mainly difficult to manage for molecular geneticists to the limited awareness of the specific traits that are linked to it. One way to overcome this problem is to identify fragments associated with factors that regulate the expression of several stress-related genes. Simple sequence repeats (SSRs) could be associated with genes of known function/s and used as functional SSR markers, thus tagging genes of interest in a more efficient manner (Hackauf and Wehling 2002).

The main objective of this investigation was to evaluate eight sugarcane clones for theirtolerance to drought stress and detect some markers associated with drought tolerance using SSR-based PCR techniques to assist selection of drought tolerant promising lines at a very early stage of the sugarcane breeding program.

MATERIALS AND METHODS

The evaluation of sugarcane germplasm one of routine work in the breeding and selection programs for producing new genotypes. In previous work in breeding and genetics department, where SCRI evaluated about 425 genotypes of sugarcane for some traits, one of them was the evaluation for drought tolerance. Depending onthese results, eight genotypes selected (i.e.G.T.54-9, G84-47, Sp 80-32-80, F.153, Co.997, BOT-41, Co.775, F.161) for this study. The origin and pedigree of these genotypes were presented in Table 1.

Two experiments were conducted (open field and sandy culture experiments) to study the performance of the eight sugarcane genotypes against drought stress and detect some morphological and molecular markers associated with drought tolerance using PCR- based techniques i.e. SSR as marker-assisted selection. The experiments carried out at the farm, greenhouse and laboratories of Breeding & Genetics department, Sugar Crops Research Institute(SCRI), Agricultural Research Center(ARC), Giza, Egypt during the period from 2012 to 2015 growing seasons.

Table 1. Names, pedigrees and origins of eight sugarcane genotypes.

Genotype name	Pedigree			Source of seed
	Male		Female	
G.T.54-9	NCO310	X	F 37-925	Seed fuzz from Taiwan
G84-47	NCO310	X	?	Local seed fuzz Official cross
Sp 80-32-80	SP71-1088	X	H575028	Sao Paulo, Brazil
F.153	NCo 310	X	P341-36	Taiwan
Co.997	Co. 683	X	P63-32	India
BOT-41	Wild sugarcane *Saccharumspontaneum*			a grass from Indonesian
Co.775	POJ 2878	X	Co.371	India
F.161	F.146	X	F.149*	Taiwan

? unknown parent

Field Experiment

The eight genotypes were planted and irrigated optimally (two times monthly with 15 days' interval) during the formative growth phase (90 days after planting). Planting date took place in the first week of March. Each genotype planted in plots containing six ridges, one meter in width and seven meters in length. The distance between cuttings was 30cm (Each cutting contained two buds) and the plot area was 42.0 m2 (6.0 m x 7.0 m). Cutting subjected for two irrigation systems as follows; control (irrigated every 15 days) and water stress (drought) treatment (irrigated every 30 days). A randomized complete block design with three replications was used. The experiment was applied for four months, then they irrigated optimally until harvest. Harvest took place thirteen months after planting. Thirty plants were taken randomly from each plot to study five yield–related traits i.e. stalk height, stalk diameter, stalk weight, leaf area and number of stalks /m^2.

Sand Culture Experiment

The eight cultivars were sown in a sand culture experiment, which was conducted according to Heakel *et al*. (1981) in plastic dishes 45 cm in height, 50 cm in diameter and capacity of 50 kg sandy soil. The plastic dishes were filled to 7 cm from the top with pre-washed fine sand. Three single bud cuttings were sown in each dish. Modified-Hoagland solution, suggested by Johnson *et al.* (1957), was used as the base nutrient solution. All sugarcane genotypes were sown in a completely randomized with three replications. Drought treatment was initiated at 21 days after sowing. Control plants were irrigated with the base nutrient solution every three days while drought stresses plants were irrigated with the base nutrient solution every two weeks. Samples were taken and data were recorded 90 days for the following yield-related traits: stalk height, stalk diameter, stalk weight, leaf area and number of stalks /m2.

Molecular genetic studies

DNA isolation

Genomic DNA was isolated from sugarcane meristem cylinder using CTAB method described by Doyle *et al.* (1987) and modified by Khaled and Esh (2008). DNA quantification was done using spectrophotometric measurement of UV absorption at wavelengths 230, 260 and 280 nm and DNA was checked by using 1% agarose gel electrophoresis (AGE)/TBE. The DNA was diluted in TE buffer to a working concentration of ~10 ng/µL.

Simple Sequence Repeats (SSR) Analysis

Eighteen SSR primers were chosen for analysis of sugarcane genotypes, twelve of them were sorghum specific primers. Primers names, sequences and annealing temperature are mentioned in Table 2. Reaction conditions were optimized and the amplification was performed for 35 cycles as follows; initial denaturation at 94ºC for 1 min (one cycle), denaturing at 94ºC for 20 sec, annealing at 50º-55ºC for 35 sec, extension at 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 electrophoresed at 90 V, in 2% agarose gel for approximately 2 h, using 0.5 × TBE buffer, along with a DNA ladder. The gel contained 0.5 µg/ml ethidium bromide to stain the DNA and photographed under UV light using gel documentation system. Reactions were duplicated to check the consistency of the amplified products.

Table 2. Primer names and their sequences for SSR-PCR analysis.

Primers		Primer sequence (5'- 3')	Primers		Primer sequence (5'- 3')
SSR9	F	AAGAAAAGGAGGGCCAAAAA	SSR12	F	AGAAGGAACGGTACCACGAC
	R	GCCAGGCAAGAGGATAAAAA		R	TTGAAGTCGAGCACGATGAG
SSR15	F	ATCCCAGAGCCCATCTCC	SSR19	F	CTTTAATCGGTTCCAGA
	R	ATCTCCATACCTCCCCAGCA		R	CTTCCACCTCCGTACTC
SSR8O	F	GTTCCCACCGCTGTCATC	SSR73	F	AACCTAAGAGGCCTATTTAACC
	R	TACGAGCACGTGTCCAACTC		R	ACGGCGACTATGTAACTCATAG

Statistical analysis

The collected data were statistically analyzed according to Bernardo (2002). Differences between means were compared using Duncan's Multiple Range Test (Duncan, 1955) and declared significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Eight sugarcane genotypes were selected (i.e.G.T.54-9, G84-47, Sp 80-32-80, F.153, Co.997, BOT-41, Co.775, F.161**)** to study their performance against drought stress and detect some morphological

and molecular markers associated with drought tolerance using PCR- based techniques.

Field and Sand Culture Experiments

The eight sugarcane genotypes were screened for drought tolerance depending on five traits (i.e. stalk height, stalk diameter, stalk weight, leaf area and number of stalks /m2). The results were presented in Table 3. All studied genotypes were significantly affected by drought in both field and sandy culture experiments with a same trend but different responses. The most affected trait was stalk weight which had the highest reduction percentage (21.55 and 28.93%), followed by No. stalk/m^2 (15.22 and 19.7 %) in field and sandy culture experiments, respectively. The highest reduction percentage was recorded for genotype Co775 with leaf area trait (39.88 %) in field experiment and stalk weight (64.44 %) in sandy culture. The results in Table 3 revealed that the most drought tolerant genotypes were Sp8032-80, Co997 and BOT41, while the most sensitive were Co775, F153 and F161. However, genotypes G84-47 and G.T.54-9 were moderate drought tolerant genotypes. Drought is the major abiotic stress that affect morphological parameters such as stalk length, stalk diameter, leaf area and number of stalks. These results agreed with those of Hemaprabha and Simon (2012), Ribeiro *et al. (*2013) and Vantini*et al.* (2015) who found that drought is an abiotic stress that limits the productivity and geographical distribution of sugarcane.

Molecular genetic studies

This investigation aimed to detect molecular markers associated with drought, tolerance or sensitivity, in sugarcane using RAPD, ISSRs, R-ISSRs and SSRs-PCR analysis

SSR Analysis:

As shown in Table 4 and Figure 1, two SSR primers produced 15 amplified fragments. One of them produced negative markers only, while the other produced both negative and positive markers. Primer SSR15 produced one band with molecular weight 123 bp which could be used as marker for drought sensitivity (negative marker), however, SSR80 produced two bands 24 bp and 35 bp that could be used as negative and positive markers. Khaled et al. (2016) evaluated SSR markers for genetic diversity analysis in sugarcane species and commercial hybrids providing valuable pedigree information which in turn is useful in progenitors choosing through breeding programs. Khaled *et al*. (2016) used SSR for genetic diversity between some sugarcane genotypes.

Table 3. Means of five yield-related traits under drought(D) for eight sugarcane genotypes compared with the control (C).

Genotype	Stalk length (cm)			Stalk diameter (cm)			Stalk weight (kg)			Leaf area (cm)2			No. stalks/m2			
	C	D	Red%	C	D	Red %	C	D	Red %	C	D	Red %	C	D	Red %	Red% mean
								Field experiment								
G.T.54-9	146.93	129.59^{G}	11.80	1.97	1.88^{D}	4.76	0.64	0.49^{F}	23.00	271.27	254.10^{C}	6.33	12.24	11.00^{D}	10.13	11.21
G.84-47	162.66	143.00^{D}	12.09	2.01	1.90^{C}	5.51	0.61	0.53^{E}	13.83	213.52	195.27^{D}	8.55	26.56	20.90^{A}	21.31	12.26
Co.997	177.94	166.95^{C}	6.18	2.11	1.90^{C}	9.82	1.08	1.05^{A}	2.21	301.07	293.37^{A}	2.56	12.32	10.45^{DE}	15.18	7.19
F.161	162.73	135.14^{E}	16.96	2.20	1.73^{E}	21.37	0.78	0.50^{F}	35.99	292.91	264.88^{B}	9.57	14.23	12.10^{C}	14.97	19.77
F.153	202.70	168.02^{B}	17.11	2.81	2.33^{A}	17.04	1.30	0.99^{B}	23.97	366.52	294.88^{A}	19.54	10.45	7.70^{G}	26.32	20.80
Co. 775	265.13	193.40^{A}	27.05	2.54	2.01^{B}	20.85	1.25	0.58^{D}	53.77	255.64	153.69^{F}	39.88	11.55	9.90^{EF}	14.29	31.17
BOT-41	193.05	167.31^{C}	13.33	2.00	1.89^{CD}	5.53	1.05	0.90^{C}	14.67	214.06	195.58^{D}	8.63	16.50	13.75^{B}	16.67	11.77
Sp 80-32-80	138.72	134.06^{F}	3.36	1.81	1.71^{F}	5.66	0.62	0.59^{D}	4.94	269.65	268.73^{B}	0.34	11.33	11.00^{D}	2.91	3.44
Mean	181.23	154.68	13.48	2.18	1.92	11.32	0.92	0.70	21.55	273.08	240.07	11.92	14.40	12.10	15.22	
								Sand culture experiment								
G.T.54-9	48.98	46.52^{E}	5.02	0.83	0.73^{FG}	12.09	0.19	0.12^{GHI}	39.39	125.95	108.90^{F}	13.54	7.51	6.60^{GH}	12.09	16.42
G.84-47	54.22	49.87^{D}	8.03	0.84	0.83^{BCD}	1.15	0.21	0.14^{EFG}	32.64	99.13	94.85^{G}	4.33	20.74	14.21^{AB}	31.46	15.52
Co.997	63.35	59.07^{A}	6.75	0.92	0.85^{B}	7.53	0.31	0.29^{A}	7.94	147.78	144.59^{A}	2.16	8.67	7.21^{E}	16.81	8.24
F.161	69.69	46.43^{E}	33.38	0.84	0.75^{E}	10.57	0.20	0.13^{FG}	34.02	132.52	126.76^{D}	4.35	9.87	8.11^{D}	17.82	20.03
F.153	67.57	57.73^{B}	14.56	1.23	1.01^{A}	18.21	0.38	0.26^{B}	31.81	170.17	141.14^{BC}	17.06	6.79	5.16^{I}	24.05	21.14
Co. 775	68.88	49.59^{D}	28.00	0.94	0.65^{I}	30.97	0.32	0.11^{GHIJK}	64.44	118.69	54.89^{JKL}	53.75	7.51	4.95^{J}	34.07	42.25
BOT-41	64.35	56.63^{BC}	12.00	0.84	0.81^{CD}	4.08	0.27	0.23^{C}	13.35	99.39	92.20^{HI}	7.23	10.73	9.08^{C}	15.38	10.41
Sp 80-32-80	46.24	43.31^{F}	6.33	0.76	0.69^{H}	8.56	0.16	0.15^{DEF}	7.87	125.20	120.93^{E}	3.41	7.36	6.93^{F}	5.90	6.41
Mean	60.41	51.14	14.26	0.90	0.79	11.64	0.25	0.18	28.93	127.35	110.53	13.23	9.90	7.78	19.70	

CONCLUSION

Analysis of SSR fragments was an effective tool for detecting marker assisted selection to improve plant breeding strategies. Applying SSRs is critical, especially in sugarcane which could be a difficult process due to the complexity of sugarcane genome. Two experiments were conducted (open field and sandy culture experiments) to study the performance of eight sugarcane genotypes under both artificial and natural condition against drought stress for assisting the most tolerant and most sensitive genotypes; and detect some markers (morphological and molecular markers) associated with drought tolerance using PCR- based techniques i.e. SSR as marker-assisted selections. It could be concluded that detecting markers based on SSRs may provide more accurate information to plant breeder. This data will help the exploitation of sugarcane germplasm on molecular basis. Future breeding efforts involving crosses between and within the groups identified in this investigation may be useful for combining beneficial genes and alleles in new sugarcane genotypes in addition to maintaining genetic diversity.

Table 4. The total number of amplified and polymorphic fragments, polymorphism % and the specific markers for drought stress in sugarcane using SSR analysis.

Primer name	Primer sequence (5'- 3')		TAF	PF	P%	SM -ve	SM +ve
SSR15	ATCCCAGAGCCCATCTCC	F	5	3	60	1	0
	ATCTCCATACCTCCCCAGCA	R				123 bp	
SSR8O	GTTCCCACCGCTGTCATC	F	10	9	90	1	1
	TACGAGCACGTGTCCAACTC	R				24 bp	35 bp
Total			15	12		2	1

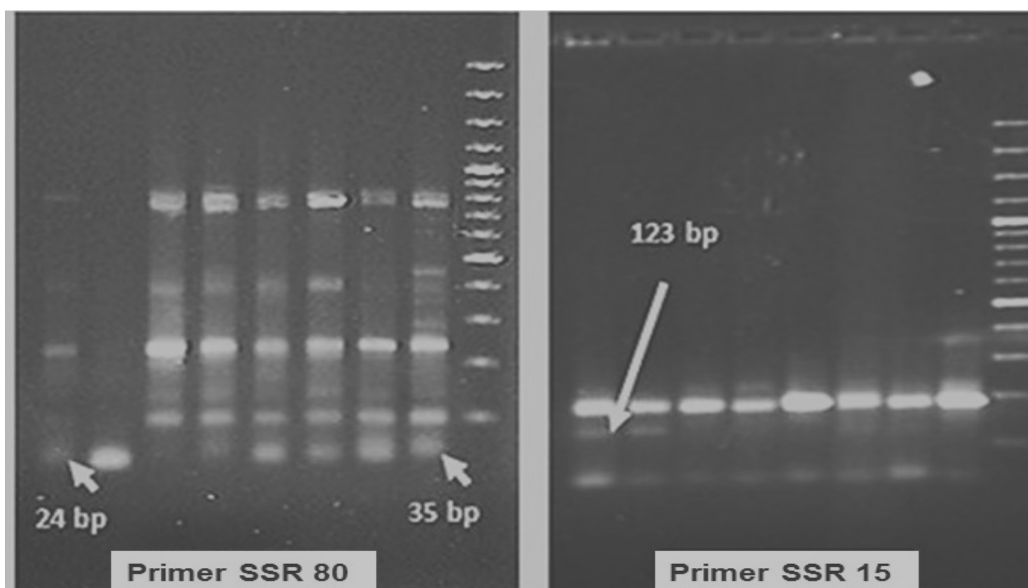

Figure 1. SSR-PCR polymorphism pattern of drought tolerant and drought sensitive clones against primers SSR9, SSR15 and SSR80; M: 100 pb plus DNA ladder, Lane1: Sp 80-32-80, Lane2: Co 997, Lane3: BOT-41, Lane4: G.T. 54-9, Lane5: G. 84-47, Lane6: F. 153, Lane7: F. 161 and Lane8: Co 775

REFERENCES

Aitken K., Jackson P., McIntyre C. (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

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

Bernardo R (2002) *Breeding for Quantitative Traits in Plants*, Stemma Press, Woodbury, MN, USA, 369 pp

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochem Bull. 19:11-15.

Duncan DB (1955) Multiple ranges and multiple F test. Biometrics 11, 1-42.

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

Fratini R, Durán Y, García P, Pérez de la Vega M (2007) Identification of quantitative trait loci (QTL) for plant structure, growth habit and yield in lentil. *Spanish Journal of Agricultural Research* 5 (3), 348-356

Hackauf, B. and Wehling, P. (2002), Identification of microsatellite polymorphisms in an expressed portion of the rye genome. Plant Breeding, 121: 17–25.

Heakel MS, El-Abasiri A, Abo-Elenin RA, Gomaa AS (1981) Studies on salt tolerance in barley and wheat. 1. Screening technique. In: Proceedings of the 4th International Barley Genetics Symposium, 22-29 July 1981, Edinburgh.

Hemaprabha, G. and Simon, S. (2012). Genetic diversity and selection among drought tolerant genotypes of sugarcane using microsatellite markers. Sugar Tech., 14 (4): 327-333.

Johnson, C.M., R. Strout, T.C. Broyer and A.B. Carlton, 1957. Comparative chlorine requirements of different plant species. *Plant Soil,* 8: 327–353

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

Khaled A M Khaled; E.A.M. Amer and A. B. A. El-Taib. 2016. Genetic Diversity of Sugarcane progenitors from the SCRI Germplasm using SSR. *J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 7 (5): 135- 139*

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 A. Khaled, N.M.S. Sbalaby and S. K. A. Ismail (2007). Molecular Genetics Characterization of QTLs Controlling Some Quality Traits in Some Sweet Sorghum Genotypes *(Sorghum bicolor*(L.) Moench). *The fifth conference of plant breeding, May 2007, 259-270*

Khaled,Shereen K.M.,FMAbdel-Tawab, EmanMFahmy and K. A. M.Khaled. 2011.Marker-Assisted SelectionAssociatedwithSugarContentinSugarcane(*Saccharum*Spp.).*Proceeding of TheThird InternationalConferenceofGeneticEngineering anditsApplications.SharmEL Sheikh City, South Sinai, Egypt, October5-8, 2011: 1-13*.

Ming, R., Del Monte, T.A., Hernandez, E., Moore, P.H., Irvine, J.E. and Paterson, A.H., 2002. Comparative analysis of QTLs affecting plant height and flowering among closely-related diploid and polyploid genomes. *Genome*, *45*(5), pp.794-803.

Ming, R., Moore, P.H., Wu, K., et al. (2006) Sugarcane improvement through breeding and biotechnology. *PlantBreeding Reviews*, 27, 15–118.

Ribeiro, R. V.; Machado, R. S.; Machado, E. C.; Machado, D. F.; Magalhães, S.P.; Filho, J. R., and Landell, M. G. A. (2013). Revealing droughtresistance and productive patterns in sugarcane genotypes by evaluating both physiological responses and stalk yield. Exp. Agric., 49, 212–224.

Silva, M.D.A., Jifon, J.L., Da Silva, J.A. and Sharma, V., 2007. Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. *Brazilian Journal of Plant Physiology*, *19*(3), pp.193-201.

Vantini ,J.S.; Dedemo ,G.C.; Gimenez ,D.F.R.; Tezza , R.I.D.; Mutton ,M.A.; Ferro , J.A. *et al*.(2015).Differential Gene Expression Between Roots of Drought-Tolerant and -Sensitive Sugar Cane Cultivars. Genet. Mol. Res. 14(2):7196-7207.

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 flammulinavelutipes. J. Biosci. Bioeng. 110, 273–275.

التتابعات المتكررة البسيطة (SSR) والمعلمات المورفولوجية المرتبطة بتحمل الجفاف في قصب السكر

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تم اختيار ثمانية طرز وراثية من قصب السكر اعتماداً على نتائج الإختبارات السابقة التي أجريت من خلال تقييم برنامج التربية بمعهد بحوث المحاصيل السكرية وهذه الطرز هي G.T.54-9, G84-47, Sp 80-32-80, F.153, Co.997, BOT-41, Co.775, F.161 وقد أجريت الدراسة بهدف الحصول على معلمات وراثية مساعدة للإنتخاب مرتبطة بتحمل الجفاف باستخدام تقنية SSR وأيضاً باستخدام بعض الصفات المرتبطة بالمحصول وهي ارتفاع وقطر ووزن الساق (النبات) ومساحة الورقةوعدد النباتات في المتر المربع. وقد تم زراعة الطرز الوراثية في تجربتين إحداهما في الحقل المفتوح والأخرى في أصص مملوءة بالرمال وذلك لتحديد الطرز المتحملة والحساسة للجفاف تحت الظروف الطبيعية وكذلك تحت ظروف الأصص. وقد أظهرت النتائج تأثر جميع الصفات تحت الدراسة بالتعرض للجفاف، فقد كانت صفة ارتفاع الساق أكثر الصفات تأثراً بالجفاف حيث سجلت أعلى نسبة انخفاض (21.55 و28.93 %) في كل من التجربة الحقلية والرملية تبعها في ذلك صفة عدد النباتات في المتر المربع (15.22 و19.7 %), كما أظهرت النتائج أن الطرز Sp8032-80 هو أكثر الطرز تحملاً للجفاف في حين كانت الطرز Co997 and BOT41 أكثرها حساسية. كما أظهرت الدراسة فاعلية تقنية SSR كمعلمات وراثية مساعدة للإنتخاب حيث تم الحصول على ثلاث معلمات يمكن استخدامهم في انتخاب الطرز المتحملة للجفاف وكانت أوزانها الجزيئية 123 bp, 24 bp and 35 bp وهذه النتائج دليل على فاعلية التقنية المستخدمة كمعلمات وراثية.