Microsatellite markers associated with salt tolerance in *Sorghum bicolor* L.

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Sara B. H. Awaly and Dalia S. Ahmed
Genetics Department and Genetic Engineering Research Center, Faculty of Agriculture,
Cairo University, Giza, Egypt.

ABSTRACT

In this study, the genetic diversification and the salinity tolerance potential of six sorghum cultivars was screened by microsatellite markers (SSR) related to salt tolerant genes. The data indicated that, the PIC (Polymorphic information content) value varies from 0 to 1 with a mean of 0.6. The SSR primers Umc1862, Umc1501 and Umc1545 showed the highest PIC values 1, 1 and 0.91 respectively, whereas Phi031 and Umc1719 showed 0.33 value. Cluster analysis based on salt tolerant gene based SSR markers, assembled cultivars into two independent clusters as tolerant and susceptible. The first cluster contains Horas and Maka-244 cultivars of highly salt tolerance, where the total scorable alleles of these genotypes was 43 and 41, respectively, while the second cluster contains Giza-420, PM, Special-85 and Special-90 cultivars of salt sensitivity, which registered 31 alleles of total scorable alleles, suggesting the prospect benefit of these SSR-markers in mapping of salinity-related traits.

Keywords: *Genetic diversification, sorghum, salt tolerance, Microsatellite, SSR markers.*

INTRODUCTION

Sorghum bicolor L. is the fifth utmost essential cultivated grain crop in the worldwide and is the third principal staple food crop after wheat and rice for billions of the poorest human in the semi-arid areas because, it has major source of proteins, energy, minerals and vitamins where, it contains 11.3% protein and 56 to 73% seeds starch (Khaton *et al*., 2016; Assem *et al.*, 2017 and Sagar *et al*., 2019). Therefore, it has various major economically utilizing like grain representing 33% feed and 55% food, ethanol as fuel, paper as fiber, methane output from fermentation and fertilizers as organic products. In addition to, the sorghum grain has elevated levels of proteins and energy to manufacture of baby foods and ice-cream cones in addition to the manufacture of feeds to poultry and animals (El Sanousi *et al*., 2016; Roy *et al*., 2018 and Sagar *et al*., 2019).

Salt stress is one of the utmost dangerous problems for plant production in the worldwide. Because it gives rise to very critical damage, which leads to decrease in germination, growth, yields, modified the physiological operations and damage of biochemical synthesis for protein, enzymes and sugars (Roy *et al*., 2018). Salinity reduction in Ca^{2+} and K^{+} and accretion of Mg^{2+} and Na^{+} ions in plant, which lead to decrease in dry matter accretion and grain production (Farooq *et al*., 2015 and Roy *et al*., 2018).

The sorghum is a moderately salinity tolerant and is recognized as a food security crop consequently it is the selected crop due to its ability to tolerate salt and drought toxicity, but it has several genotypic variety for salt tolerance (Sun *et al*., 2014 and Amelework *et al*., 2015). So, the evolution of the salt tolerant sorghum cultivars is necessary for increase productivity in salt stress for efficient strategy of plant improvement (El Sanousi *et al*. 2016 and Krupa *et al*., 2017).Salt tolerance is a polygenic attribute. Therefore, one of the strategies to sorghum improving depends on screening sorghum genotypes by molecular marker technology to determine salinity tolerance genes to develop new tolerant cultivars. DNA based marker utilized to tag quantitative trait loci (QTL) by choosing suitable alleles at those loci and to estimate their assistance to the phenotype to increase genetic improvement (Rani and Sharma, 2019). One of these markers is SSRs (simple sequence repeats) or microsatellite markers, which are highly polytrophic, co-dominant inheritance, multi-allelic, reproducible, plentiful and disseminated into the genome (Ahmadpour *et al*., 2017 and Aljumaili *et al*., 2018). This marker appears huge range of allelic variation due to difference in the number of the repeat units and diversity in the length of SSRs by utilizing specific primers to the unique flanking sequences (Rani and Sharma, 2019).The SSR markers are widely applied to locate the genes associated with salinity tolerance and genome mapping (Rahman *et al.,* 2019). Thus, SSR marker plays a significant role in determining the fundamental genes linked to salinity tolerance that are beneficial for plant breeders to determine the salinity tolerant cultivars, which are novel exporter for future plant breeding strategies (El-Hendawy *et al*., 2019).

The aim of study was to indicate the power of using microsatellite markers associated with different salt tolerant loci that are distributed across the genome of six sorghum cultivars. The genotypic diversity and cluster analysis was performed among the different sorghum genotypes used.

MATERIALS AND METHODS

In this study six sorghum cultivars namely Horas, Maka-244, Giza -420, PM, Special-85 and Special-90 were used. Seeds were collected from the Sorghum Department, Field Crops Research Institute, ARC, Giza, Egypt. Seeds were germinated in the field in order to collect young leaf samples. Extraction of DNA from young leaf samples was performed using the CTAB (cetyltrimethyl ammonium bromide) method according to Rogers and Bendich (1985); 0.5 g of leaf homogenate was extracted with pre-heated 2% CTAB buffer including100 mM Tris-HCI (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 0.1% of 2-mercaptoethanol, then incubated at 60° C for 1h. Sequentially, added equal volume of chloroform and centrifuged at 12000 rpm for 5 min., then the aqueous phase were transferred into new tubes and 3 volumes of cold 95% ethanol was added to precipitate the DNA. The blend was incubated on -20ºC at overnight, then centrifuged at 12000 rpm for 5 min. The pellet was washed with 70% ethanol, air-dried and dissolved in 30 µl H_2O. DNA quality was checked on 1 % agarose gel stained with ethidium bromide.

SSR assay

Twelve SSR primers related to salinity tolerance were selected according to Shiri (2011). Table 1 revealed the name of Primers, sequences and annealing temp. and the reaction of PCR was accomplished in 20 µl consisting of 1 µL of DNA as template (50 ng), 1 µL of forward primers (10 pmol),1 µL of reverse primers (10 pmol), 10 µL Master

Mix (GeneDireX) and 7 µL d.H2O. Amplification was accomplished in a thermo-cycler (Biometra, Germany) utilizing the following program consisting of 94ºC for 5 min. to initial DNA denaturation, sequentially 30 cycles each containing of 94 °C for 1 min, 58.4-66 °C for 1min. and 72 °C for 1 min and lastly 72 °C for 5 min. The product of PCR was investigated in a 1.2% agarose gel.

Table (1): List of the SSR primers used for molecular characterization and annealing temperature.

No.	Name of primer	Repeats		Primer Sequence 5'-3'	Anneal temp.
1	**Umc1862**	(GA)8	F	ATGGGCACATGAAAAAGAGACATT	**62ºC**
			R	CCCATGAGAAGAGTGAAGACAACA	
2	**Umc1501**	(AAG)5	F	CCACATTTGGCTGAATTTGTTGTA	**61ºC**
			R	CTTGTTGGCTAGAAATTTGCCTTG	
3	**Umc1333**	(CAG)4	F	AGGTAAGCGAGCATCTGAGGGT	**64.5 ºC**
			R	TCTGGAGACTCTTCTGGGTGAACT	
4	**Umc1545**	(AAGA)4	F	GAAAACTGCATCAACAACAAGCTG	**61 ºC**
			R	ATTGGTTGGTTCTTGCTTCCATTA	
5	**NC133**	GTGTC	F	AATCAAACACACACCTTGCG	**57.5 ºC**
			R	GCAAGGGAATAAGGTGACGA	
6	**Phi031**	GTAC	F	GCAACAGGTTACATGAGCTGACGA	**64.5 ºC**
			R	CCAGCGTGCTGTTCCAGTAGTT	
7	**Phi080**	AGGAG	F	CACCCGATGCAACTTGCGTAGA	**64 ºC**
			R	TCGTCACGTTCCACGACATCAC	
8	**Bnlg1617**	AG(16)	F	CGTGCACGGTACAGAAAGAA	**58.4 ºC**
			R	AGAAAGCCACGTACCCCTTT	
9	**Umc2359**	(AAAAG)4	F	CTGGATCAGATGAAAAAGAAGGGA	**63 ºC**
			R	GCCTGACATGAATGTTACATGAGC	
10	**Umc1719**	(GCG)5	F	CCTGGAAGCACCACTGATACTAGC	**66 ºC**
			R	AGCTCCAGCCTGCCTACCAG	
11	**Umc1447**	(CTT)4	F	TAATACTCCTACTAACGGCGCTGC	**63 ºC**
			R	TCTGTCTCCCATGCCTGAAATAAT	
12	**Umc1432**	(AG)6	F	GGCCATGATACAGCAAGAAATGAT	**63ºC**
			R	TACTAGATGATGACTGACCCAGCG	

SSR Markers data analysis

The various alleles were scored based on presence or absence of the SSR bands in various SSR primers and the molecular weight of each band by DNA ladder was determined. The Multi Variate Statistical Package (MVSP) version 3.22 genetic analysis software was used to create the dendrogram among sorghum cultivars based on Jaccard's similarity coefficient (Jaccard, 1980) and UPGMA method created from Nei's (1983).

RESULTS AND DISCUSSION

High concentration of salts is the fundamental restriction for plant growth, development and productivity, because it has injurious and toxic effects on growth, yield and morphological stages, where it reduced metabolism, protein synthesis and water absorption by roots (Tariq *et al.,* 2019).Molecular markers such as microsatellites or SSR (simple sequence repeat) were used to evaluate the genetic diversification, identification and

characterization of genes responsible for the attribute of serious like salt stress to evolve the effective selection of this intricate attributes (Meyer *et al.,* 2017; Ishikawa *et al.,* 2018 and Elshafei *et al*., 2019). Therefore, Marker assisted selection (MAS) such as SSRs were used to develop genotypes possessing appropriate abiotic stress tolerance attributes by selected-associated markers. Abiotic stress tolerance candidate genes such as salt stress responsive genes are significant objectives for determination of SSR markers, which can be effectively and efficiently utilized for mapping of abiotic stress tolerance attributes. So, gene based SSR markers evolved from salt responsive candidate genes would be extremely beneficial for relationship mapping of salt tolerant related attributes in plants (Goyal *et al*., 2016; Singh *et al.,* 2018 and Wang and Xia, 2018).In this study, salt stress tolerant genes were screened through the use of twelve SSR markers according to Shiri (2011) in order to evaluate the salt tolerance differences among the six sorghum genotypes to distinguish salt tolerant sorghum genotypes to obtain new source of salt tolerance for future breeding programs. All SSR-primers generated fragments and appeared various levels of polymorphism (Fig. 1).

The twelve SSR primer pairs were applied to assay their differentiation power (DP) by accounting the PIC of their loci, showed in Table 2. The amplicon size of the revealed alleles generated ranged from 90 to 1500 bp that revealed a high variety in the number of repeats between the various alleles. The Umc1545 and Umc1447 markers showed the highest allelic numbers (11 and 10, respectively), whereas, the Umc1333, Bnlg1617 and Umc2359 revealed 2 alleles as the lowest allelic numbers. The percentage of polymorphism or PIC value (Polymorphic Information Content) varies from 0 to 1 with a mean of 0.6. The Umc1862, Umc1501 and Umc1545 showed the highest value of PIC (1, 1 and 0.91 value, respectively), whereas Phi 031 and Umc1719revealed 0.33 value, but the Bnlg1617 and Umc2359 detected no Polymorphism among cultivars (PIC = 0).

Table (2): List of the SSR primers used for molecular characterization, allele no., and PIC (Polymorphism information content).

No.	Name of primer	Repeats	Total alleles	Polymorphic alleles	PIC	Amplicon size range (bp)
1	Umc1862	(GA)8	7	7	1	350-90
2	Umc1501	(AAG)5	3	3	1	260-100
3	Umc1333	(CAG)4	2	1	0.5	250-90
4	Umc1545	(AAGA)4	11	10	0.91	1000-150
5	NC133	GTGTC	4	3	0.75	500-90
6	Phi031	GTAC	3	1	0.33	300-100
7	Phi080	AGGAG	5	4	0.8	1000-150
8	Bnlg1617	AG(16)	2	0	0.00	180-90
9	Umc2359	(AAAAG)4	2	0	0.00	100-90
10	Umc1719	(GCG)5	3	1	0.33	750-150
11	Umc1447	(CTT)4	10	7	0.7	1500-100
12	Umc1432	(AG)6	6	5	0.83	1400-100
	Total		58	42	7.15	
	Mean		4.8		0.6	

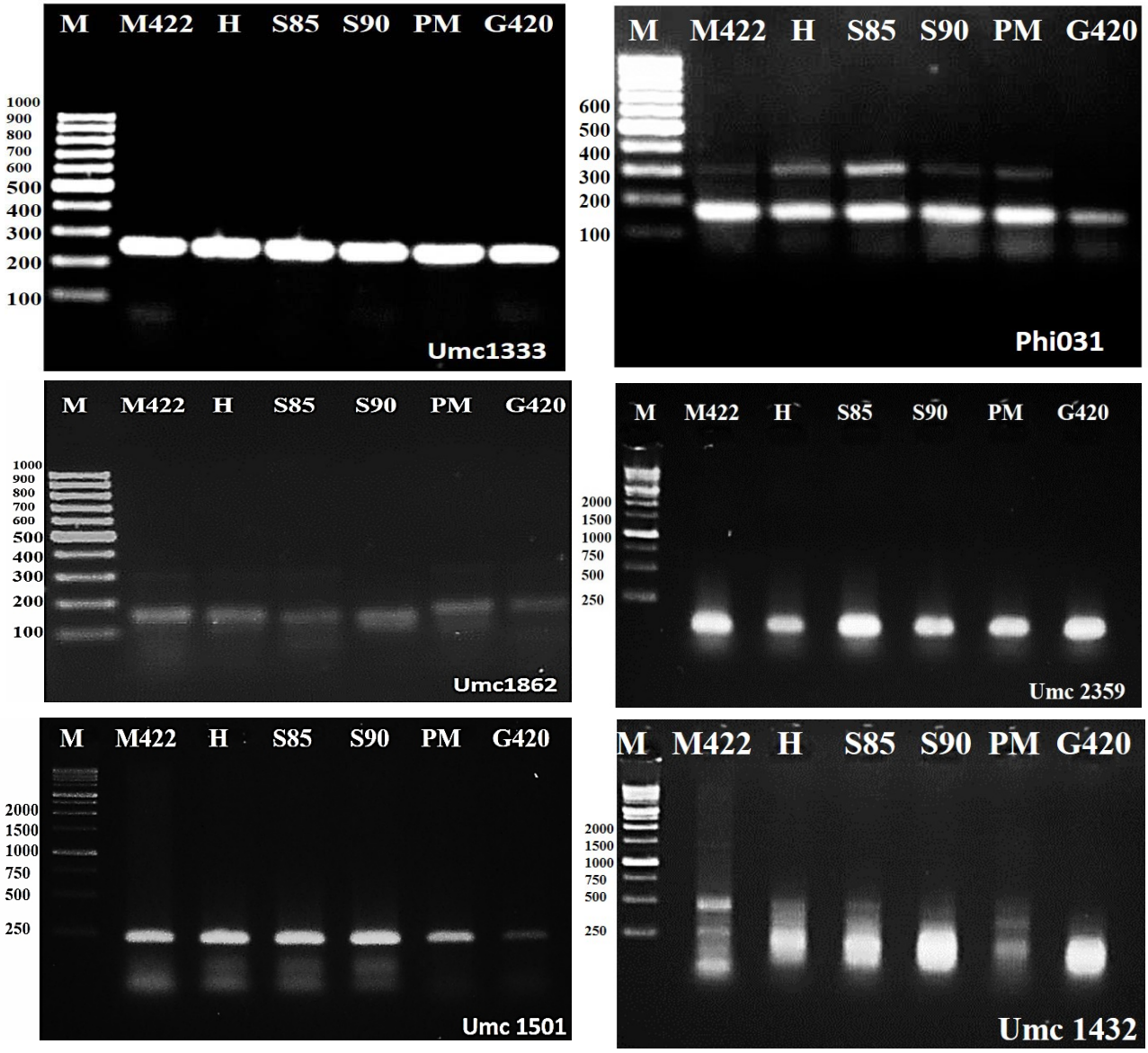

Fig. (1). SSR banding patterns of six different sorghum genotypes ((M244) Maka-244, (H) Horas, (S85) Special-85, (S90) Special-90, PM and (G420) Giza -420), (M) 100bp and 1 kbp plus DNA ladder.

Table 3 shows the genotype specific SSR markers for the six sorghum cultivars.The data indicates that, eleven out of forty two polymorphic markers produced were found to be genotype specific, which representing 26.2%. The Special-85 cultivar revealed 5 specific markers as the highest number, followed by Maka-244 showed 3 markers then Giza -420 detected two markers, whereas, Horas revealed one marker.

Table (3): Genotype specific-SSR marker and total scorable alleles for sorghum cultivars.

Genotypes	SSR markers		Total marker
	1	0	
Maka-244	Umc1862 (90) Umc1447 (1300) Umc1432 (1400)	-	3
Horas	Umc1545 (1000)	-	1
Special-85	Umc1862 (100) NC133 (90) Phi080 (500)	Umc1545 (150) NC133 (100)	5
Special-90	-	-	-
PM	-	-	-
Giza -420	Umc1545 (650)	Phi031 (300)	2
Total			11

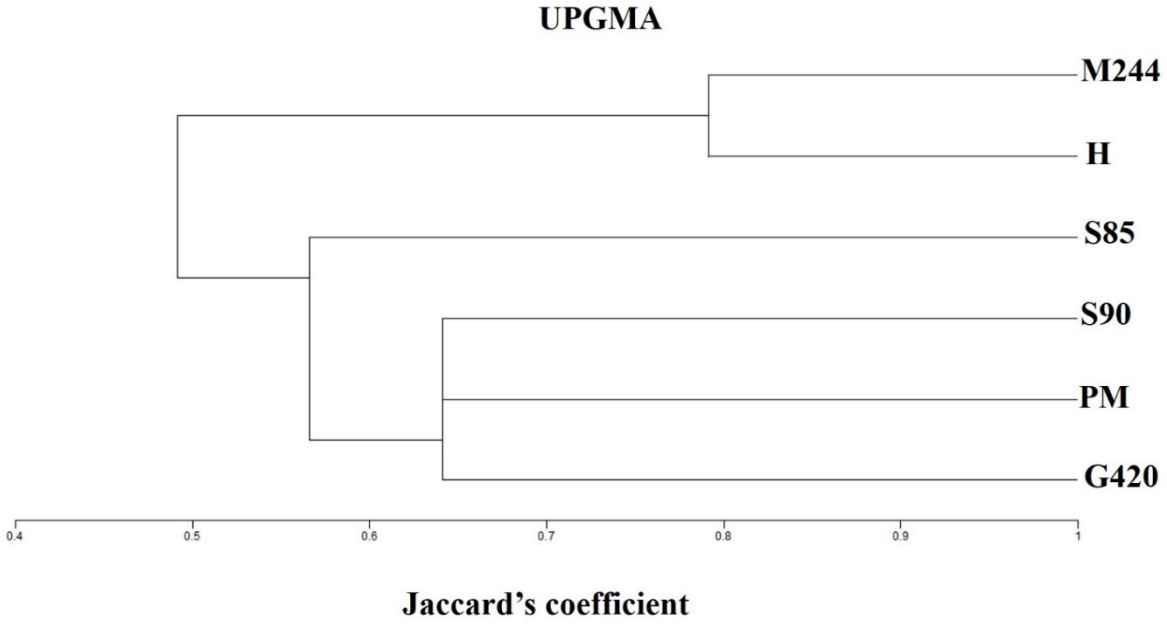

Fig. (2): Dendrogram of 6 sorghum genotypes ((M244) Maka-244, (H) Horas, (S85) Special-85, (S90) Special-90, PM and (G420) Giza -420) based on 12 polymorphic SSR markers according to the un-weighted pair group mean algorithm (UPGMA) with the Jaccard's similarity index.

The Jaccard's coefficient and the UPGMA (Unweighted pair-group method) was used to produce dendrogram based on 12 SSR markers and six sorghum cultivars to identify the genetic divergence related to salt toleranceas shown in Figure 2. Therefore, different factors like SSR loci and repeat types affect allelic variations (Tahir and Maeruf, 2016) so, this result showed two significantly various groups of salt tolerant and sensitive cultivars into independent clusters. Where, the first cluster contains H and M244 cultivars of highly salt tolerant, where the total scorable alleles of these genotypes was 43 and 41, respectively (Fig. 3). The second cluster contains S85, S90, PM and G420 cultivar which are salt sensitive and recorded 31 alleles of total scorable alleles, suggesting the prospect benefit of these SSR-markers in mapping of salinity-related traits.

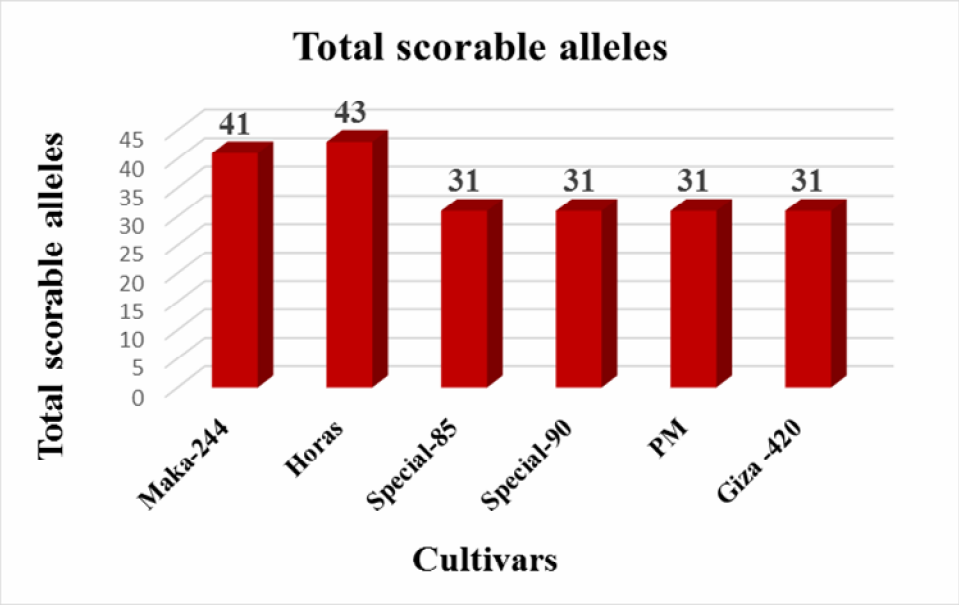

Fig.(3): Allelic frequencies differ among six sorghum cultivars as revealed by SSR analysis.

Similarly, Moghaieb *et al.* (2011) evaluated the genetic diversity and relationships between nine wheat cultivars for their salt tolerance using SSR and RAPD markers. Shiri (2011) evaluated the genetic relationships for drought tolerance in the 38 maize hybrids and identified informative 12 microsatellite markers for abiotic stress tolerance. Gamar *et al.* (2013) evaluated 95 sorghum cultivars using 39 SSRs markers to screen the genome of sorghum. Tahir and Maeruf (2016) estimated the genetic diversification for salinity tolerance in nine maize cultivars using 18 SSR markers. Singh *et al.* (2018) used 161 SSR markers for 94 salinity tolerance candidate genes of wheat. These SSR markers showed grouping of salt oversensitive and tolerant genotypes independent clusters, indicating their power useful in genetic mapping evaluation. Elshafei *et al.* (2019) evaluated eleven bread wheat cultivars for their agronomic attributes under salinity stress using 33 SSR primers, therefore cluster analysis based on the SSR results separated the 11 wheat cultivars into three groups.

CONCLUSION

SSRs markers associated with salt responsive genes were used to reveal their usefulness in distinguishing functional variation among salt sensitive and tolerant sorghum cultivars. The results revealed relationship between sorghum cultivars with SSR markers, so these markers can be beneficial in sorghum selection for salinity tolerance. The six sorghum cultivars can be divided into two significantly various groups of salt tolerant and sensitive cultivars into independent clusters. Thus, SSR analysis is able to reveal main gene locus and identify salt tolerant sorghum for sorghum breeders to improve novel genotypes that can be distributed under saline environment.

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الملخص العربي

الواسمات الجزيئية SSR المرتبطة بتحمل الملوحة في الذرة الرفيعة *Sorghum bicolor* L.

سارة بيومي حسن عوالي - داليا سيد احمد

مركز بحوث و دراسات الهندسة الوراثية – قسم الوراثة –كلية الزراعة –جامعة القاهرة

تم دراسة التنوع الوراثي ومدي تحمل الملوحة لستة أصناف من الذرة الرفيعة بأستخدام الواسمات الجزيئية microsatellite markers (SSR) المرتبطة بالجينات المتحملة للملوحة. وأوضحت النتائج أن نسبة تعدد المظاهر بين الأصناف تتراوح من صفر إلي ١ بمتوسط ٠.٦ . حيث أظهرت البادئات Umc1862, Umc1501 and Umc1545 أعلي نسبة لتعدد المظاهر وهي ١ و ١ و ٠.٩١ على التوالي ، بينما أظهرت البادئات Phi031 and Umc1719 نسبة ٠.٣٣. وتم تحليل البيانات ورسم شجرة القرابه بناءا علي تحمل الملوحة حيث انقسمت الاصناف الي مجموعتين مستقلتين المجموعة الأولي تحتوي علي الأصناف المتحملة للملوحة وهي Horas و Maka-244 حيث سجلت هذه الأصناف أعلي عدد من الأليلات وهي ٤٣ و ٤١ ، على التوالي ، بينما تحتوي المجموعة الثانية علي الأصناف الحساسة للملوحة وهي Giza-420 و PM و Special-85 و Special-90 حيث سجلت جميعها ٣١ أليل . وهذا يوضح مدي فائدة الواسمات الجزيئية في رسم الخرائط الوراثيه للصفات المرتبطه بالملوحة .