Assess The Degree Of Genetic Divergence Among Sixteen Complexes Genetically Wheat Bread Using Ssr Indicators

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Abstract: laboratory study was carried out to ; (1) identify the efficient primer(s) which can be used to detect alleles,(2) estimate the degree of diversity among the studied genotypes, and (3) determinate the suitable genotypes which show high degree of diversity in order to improve the economic characters in the local genotypes. The present study was conduct at Nebraska University, Field Crop & Horticulture Department during summer 2013. Ten selected simple sequence repeat (SSR) marker sets were evaluated in a total of sixteen accessions of bread wheat (*Triticum aestivum*), twelve of which have been introduced from Mexico, two from USA and two were local genotypes (Latifya and Ibah 95).All the 10 markers were polymorphic and produced 45 alleles (average 4.5). The gmw480 marker had higher percentage of frequency, while xgwm132 and gmw32 markers had lowest percentage of frequency, therefore they showed high efficiency in categorizing the studied genotypes. The PIC values were 0.32 for gmw480 and 0.99 for xgwm132 & gmw32 and gmw 32 markers (average 0.86). The dissimilarity coefficient (diversity) ranged between 0.2 for BW3-9830& BW3-9832 genotypes and 1.0 for many genotypes (average 0.80). The highest dissimilarity coefficient for the introduced genotypes was 0.97 for genotype BW49398 while the lowest value was 0.71 for genotype CW15-6732. According to the cluster analysis, the studied genotypes distributed into two major groups. The group I and II consist of four and three sub-groups respectively. A recommendation was made to take advantage of the divergent genotypes in the breeding program.

Index Terms: Wheat, Divergence, SSR

1 INTRODUCTION

Wbread (Triticum aestivum) is one of the most prevalent species of wheat around the world, as an area of 225,437,694 hectares are planted around the globe at a rate of production of 3.02 tons $h a^{-1}$[9]. The wheat bread has a six fold genome makes it high diversity and can spreads in most parts of the globe. This diversity opens the way for plant breeders to produce convoy genotypes for climate changes and population growth. The exclusive use of local genotypes makes the work difficult for the breeder, so should interest in introduction of elite and new genetic materials to constantly raise productivity and quality of the characteristics for the local genotypes. Introduction of these genotypes should be preceded by an estimation of genetic divergence level about the local genotypes before introducing them into various breeding programs in order to not be working at random. Genetic variation depends on complex statistical methods which rely on the variation among the alleles for a particular site locus as well as among multiple allelic sites. The genetic divergence can be assess in different ways, such as (i) Phenotypic parameters, which are characterized by high response to the environment as well as the difficulty of obtaining associated parameters with the economic traits,(ii) Isozyme which depends sometimes on particular tissue or defined growth stage,(iii) Genetic origin(pedigree).One of most efficient means that used for estimation the degree of genetic divergence is (iiii) molecular techniques.

The most important of these techniques that used in the plant and the most prevalent technology is simple repetitive sequences (Simple Sequence Repeat = SSR) or Microsatellite. This technology is based on the diagnosis of 2-6 b.p. of repeated sequences [5, 10, 27] or 1-10 b.p. [3] such as (TG) n or (AAT) n and others. These parameters spread widely across eukaryotic organism's genome (humans, animals and plants) and are frequently high covariance [13] .SSR markers assisting in classification of the genotypes through identification the differences in the number and locations of the repeated sequences. There is no information about the origin of this marker, but it is believed to be resulting from irregular genetic crossing over among the frequent units during meiosis [17], while doubling of DNA is responsible for the differences among them [19]. Microsatellite is characterized as having high polymorphism, has a capacity to produce varied bonds significantly compared to other markers, and is co-dominance. The most important uses of SSR is to estimate the diversity , genetic mapping and fingerprinting [11,12], also it could provides a model for estimation the variation among isolated generations [16] and assists in improvement of crops efficiently through diagnosis favorite alleles and collecting them. SSR markers have employed by many researchers in the genetic diversity studies of various crops, mainly wheat [1,4 ,6,7 ,8,14, 15,20,21 ,24,25,28, and 30]. Many studies have shown that SSR alleles located in genome A and B compared to D [22, 29]. This disagreement over the distribution of these alleles across the genome may effect in the classification of the genotypes [20]. The current study aims to (1) determinate the efficient primer (s) in the allelic diagnosis (2) estimate the degree of diversity among the studied genotypes, and (3) determinate the appropriate genotype that possess high degree of diversity to improve its traits through plant breeding programs.

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2 MATERIALS AND METHODS

2.1 plant material

Fourteen genotypes of wheat bread (Triticum aestivum) ,which were entered from USA & Mexico with two Iraqi genotypes (M = Latifya and N = Ibah95) (Table 1), have used with ten primers(Table 2) in order to estimate the degree of genetic divergence using SSR technology. The current study has conducted at the department of field crops and horticultural, University of Nebraska during the year 2013.

2.2 DNA Isolated

The seeds have sown in pots under laboratory conditions (15-20 $\mathcal{C}^{\circ}$). The leaflets have collected for each genotype at stage of two leaflets and kept in sterile tubes, thereafter have crushed by a Tissue Lyser machine for five minutes. DNA was extracted according to Shaha and others [23]. The concentration and purity of DNA have estimated using a Thermo Scientific Nano Drop Spectrophotometer and the values of the wavelength range 260/280 were between 1.73 to 2.14 ul / ng. The Concentrations were modified by adding a solution TE.

Table1. The pedigree of the studied genotypes

Genotype	Symbol	Origin	Pedigree
BW3-9830	A	Mexico	SIDS 10/CIRCUS/7/CMH79A.955/5/AGA/4/4*PI/CHR//2*SN64/3/INIA66/6/NAC
BW3-9872	B	Mexico	CMH82A.1294/CMH84.3621//CMH81.749/3/ELVIRA
BW3-9832	C	Mexico	CMH79A.955/5/AGA/4/4*PI/CHR//2*SN64/3/INIA66/6/NAC*2/7/ELVIRA
BW3-9852	D	Mexico	TEG/GANFRENCH
CW15-6723	E	Mexico	IWA 8607408
CW15-6732	F	Mexico	IWA 8607419
BW3-9871	G	Mexico	TEG//CMH82A.1294/CMH84.3621/3/ELVIRA
CW15-6722	H	Mexico	IWA 8607407
BW4-9475	I	Mexico	WAXWING*2/HEILO
BW4-9286	J	Mexico	UP2338*2/KKTS*2//YANAC
BW4-9082	K	Mexico	ROLF07*2/5/FCT/3/GOV/AZ//MUS/4/DOVE/BUC
BW4-9398	L	Mexico	ALTAR84/AE.SQUARROSA(221)//3*BORL95/3/URES/JUN//KAUZ/4/WBLL1/5/MILAN/S87230//BAV92
LATIFYA	M	Iraq	Australian line x Aras (Hybridization) →(selection)
IBAH-95	N	Iraq	((Veery S))
GOLDEN 86	O	USA	Plainsman V / Kodiak Dwarf / Goertzen 3028
KLASIC	P	USA	Klein Rendidor / 2*Sonora 64 /2/ Inia 66 /3/ Ciano 67 /4/ Yecora 70

2.3Polymerase chain Reaction (PCR)

PCR reaction was carried out using a thermal cycler and the reaction mixture (volume 25 µl) consisted of 10.65 water, 2.5 µl dNTP , 2.5 µl solution pcr , 2.o µl Mgcl2 , 0.35 µl Taq polymerase , 3.0 µl DNA and 4.0 µl of each primer . Table 3 shows the temperatures and periods for each step of pcr reaction. PCR products were separated at the electrophoresis for 120 minutes on polyaccrylamide gel, which was prepared

Table2. The SSR primers with their sequences

Primer	Sequence
wmc153-F	5-ATGAGGACTCGAAGCTTGGC-3
wmc153-R	5-CTGAGCTTTTGCGCGTTGAC-3
wmc264-F	5-CTCCATCTATTGAGCGAAGGTT-3
wmc264-R	5-CAAGATGAAGCTCATGCAAGTG-3
wmc169-F	5-TACCCGAATCTGGAAAATCAAT-3
wmc169-R	5-TGGAAGCTTGCTAACTTTGGAG-3
Xohg471-F	5-TGGATTTGATGGCGGAGACC-3
Xohg471-R	5-CAAGACTGACAACACAAGAC-3
wmc532-F	5-GATACATCAAGATCGTGCCAAA-3
wmc532-R	5-GGGAGAAATCATTAACGAAGGG-3
cfa2193-F	5-ACATGTGATGTGCGGTCATT-3
cfa2193-R	5-TCCTCAGAACCCCATTCTTG-3
xgwm132-F	5-ATCTAAACAAGACGGCGGTG-3
xgwm132-R	5-ATCTGTGACAACCGGTGAGA-3
gmw32-F	5-TGCTTGGTCTTGAGCATCAC-3
gmw32-R	5-TATGCCGAATTTGTGGACAA-3
gmw391-F	5-ATGTGCATGTCGGACGC-3
gmw391-R	5-ATAGCGAAGTCTCCCTACTCCA-3
gmw480-F	5-CCGAATTGTCCGCCATAG-3
gmw480-R	5-TGCTGCTACTTGTACAGAGGAC-3

from the following components: 20 ml of urinary Acrolamayd 12% ,165 µl solution of APS 20% and 1.5 ml solution TEMED. The products of electrophoresis have treated with ethylene bromide dye for fifteen minutes then soaked in distilled water for half an hour. The bonds have examined by UV short wave in a UV pro-transiluminator and have photographed by a unit Imager Gel Documents.

Table 3. PCR program

Step	Temperature ($\mathcal{C}^{\circ}$)	Period (minutes)
1	94	3
2	94	0.45
3	55	1
4	72	1
5	Repeat stage 2 foe 34 time ,then go to stage 6	-
6	72	
7	4	4

2.4 Data scoring and data analysis

For statistical analysis, the formed bonds per primer were considered as an allelic site (locus) and then set up a matrix (binary) for the genetic diversity which has used to create a dendogram and estimate the relationship among the studied genotypes by R3.01 program. The values of PIC (polymorphism information content) have calculated according to Bostein and others [2].

$$PIC=1-\sum Pij2$$

Where P: is the repeat of allele through all the genotypes. The degree of genetic divergence has estimated according to Nei and Li [18].

3 Results

All the primers have produced variant bonds through the studied genotypes, as a result 45 allele sites have been produced at a rate of 4.5 and ranged from 1 for xgwm132 and gmw32 (in genotypes KLASIC and IBAH95 respectively) to 7 sites for wmc153 and wmc264 (appeared in all genotypes except for BW3-9830, BW3-9872, BW3-9832, CW15-6723 and BW49398). Genotype BW49475 has had a highest number of alleles reached to 11 alleles across all the primers except xgwm132, gmw32 and gmw391, while genotype BW49082 has had the fewest alleles reached 3 which have diagnosed by the primers wmc153 and wmc264.The primer gmw480 owned a highest repetition, where it identified alleles in all the genotypes except BW3-9832, BW4-9398 and LATIFYA, while the primer xgwm132 gmw32 owned the lowest percentage of repeat alleles, where it identified one allele in two genotypes. PIC values has estimated for the studied primers on the basis of their performances across the sixteen genotypes, which ranged between 0.32 for gmw480 primer to 0.99 for xgwm132 and gmw32 primers at a rate of 0.86 (Table 5). The values of diversity coefficient ranged between 0.2 for BW3-9830 and BW3-9832genotypes to 1.0 for number of genotypes at a rate of 0.80 (Table 4). The local genotype LATIFYA showed high rate of genetic diversity (0.95) while the other local genotype, IBAH95, showed a moderate rate of genetic diversity (0.75). This refers to high probability of improving genotype LATIFYA as compared with genotype IBAH95 from the studied genotypes, therefore the highest

Table 4. Values of genetic diversity for the genotypes studied

CW15-6722	BW3-9871	CW15-6732	CW15-6723	BW3-9852	BW3-9832	BW3-9872	BW3-9830	
0.83	0.84	0.81	0.80	0.81		0.4	0	BW3-9830
0.8	0.81	0.77	0.75	0.77	0.25	0		BW3-9872
0.8	0.81	0.77	0.75	0.77	0			BW3-9832
0.81	0.83	0.4	0.77	0				BW3-9852
0.8	0.81	0.55	0					CW15-6723
0.81	0.83	0						CW15-6732
0.38	0							BW3-9871
0								CW15-6722

Table 4. continued

KLASIC	GOLDEN 86	IBAH-95	LATIFYA	BW4-9398	BW4-9082	BW4-9286	BW4-9475	
1	0.66	0.69	1	1	0.77	0.8	0.88	BW3-9830
1	0.8	0.63	1	1	0.71	0.75	0.86	BW3-9872
1	0.6	0.63	1	1	0.71	0.75	0.86	BW3-9832
69	0.63	0.5	1	1	0.75	0.77	0.62	BW3-9852
0.5	0.8	0.81	1	1	0.71	0.75	0.73	CW15-6723
0.53	0.45	0.5	1	1	0.75	0.77	0.75	CW15-6732
1	0.84	0.85	1	1	0.8	0.81	0.88	BW3-

Table5. Values of PIC and Number of Alleles for each Primer

Primer	PIC	Allele Numbers
wmc153	0.87	7
wmc264	0.85	6
wmc169	0.86	7
Xohg471	0.87	6
wmc532	0.96	5
cfa2193	0.92	6
xgwm132	0.99	1
gmw32	0.99	1
gmw391	0.97	4
gmw480	0.32	2

Degree of genetic diversity of genotype LATIFYA happened with most of the studied genotypes, while the same degree of genetic diversity happened between genotype IBAH95 and only two genotypes. Fig. 1 shows the results of cluster analysis, which has classified the genotypes into two groups depending on the basis of their performances across ten primers. The genotypes distributed into two groups; group I consisted of four subsets, and group II consisted of three subsets. Group I consisted of BW3-9830, BW3-9872, BW3-9832 , BW3-9871, CW15-6722, BW49286, BW49082 , BW49398 and LATIFYA, while Group II included BW3-9852, CW15-6723, CW15-6732, BW49475, IBAH95, GOLDEN 86 and KLASIC.

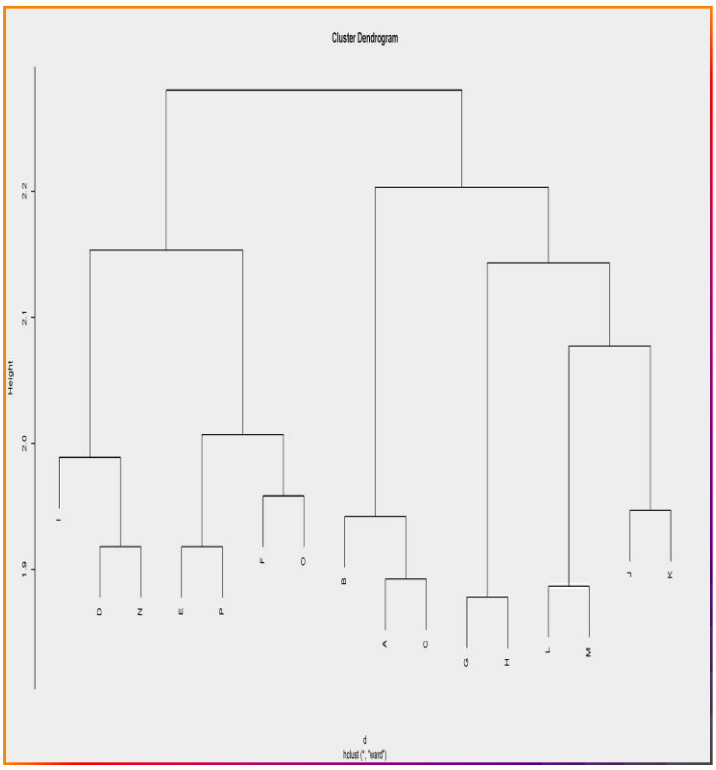

Figure1. The Cluster Analysis of the wheat genotypes based on them performances through ten Primers

4 Discussion

PIC values provide an estimate of the efficiency of allelic site in the classification of the studied genotypes and its values ranging between 0 (monomorphic) to 1 (high polymorphic). The PIC values obtained in this study were high as compared to the values from previous studies, taking into account the number of the studied genotypes. The values of diversity coefficient ranged between 0.2 for BW3-9830 and BW3-9832 genotypes to 1.0 for number of genotypes at a rate of 0.80. That rate refers to the existence of genetic diversity among the studied genotypes. Note that the values of PIC refer to the extent of divergence or convergence of the genotypes only for the used primers, therefore using another set of primers may gives different degrees of divergence, hence use of a wide range of primers is necessary for screening them and define the efficient one. The highest rate of genetic diversity reached 0.97 for genotype BW49398, while the lowest rate was 0.71 for genotype CW15-6732. We note that the genotype BW49082 and in spite of own the lowest number of alleles which was diagnosed using the studied primers, but it did not own the highest rate of the genetic diversity value, that is because genotype BW49082 identified the same alleles which have been identified by number of genotypes. We can conclude that the number of diagnosed alleles is not the most important factor in estimation the degree of genetic diversity. The genotypes distributed into two groups; group I consisted of four subsets, and group II consisted of three subsets. Note that the genotypes LATIFYA and IBAH95 have located in

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