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# Assess the Degree of Genetic Divergence among Sixteen Complex Genetically Wheat Bread using Indicators SSR

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***Abstract:*** *A laboratory study was carried out to; (1) identify the efficient primer(s) which 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 for the local genotypes. The present study was conduct at Nebraska University/ Field Crop & Horticulture Department during summer season 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 are local genotypes (Latifya and Ibah 95).All the 10 markers were polymorphic and produced 45 alleles (average 4.5). The gmw480marker had the higher percentage of frequency, whilexgwm132 and gmw32 markers had the lowest percentage of frequency, therefore they showed high efficient in categorizing the genotypes studied. The PIC values were 0.32 for gmw480 and 0.99 for xgwm132 & gmw32markers (average 0.86). The dissimilarity coefficient (diversity) ranged between 0.2- 0.99(average 0.80). The highest dissimilarity coefficient for the introduced genotypes was 0.97 for BW49398 genotype while the lowest value was 0.71 for CW15-6732 genotype. 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.*
***Keyword:*** *Wheat, SSR, Divergence.*

## I. Introduction

Wheat bread (*Triticumaestivum*) is one of the most prevalent species of wheat around the world, as an area of 225437694 hectares are planted around the globe at a rate of production of 3.02 tons$ha^{-1}$(FAO, 2013). Wheat bread has 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 plant breeder, so it should be interest in the introduction of elite and new genetic material to constantly raise productivity and quality characteristics of the local genotypes. Introduction of these genotypes should be preceded by an estimation of the value of genetic divergence regarding to the local genotypes in order to avoid random work. Genetic variation depends on complex statistical methods which rely on the variation among alleles for a particular site locus as well as among multiple allelic sites. The genetic divergence can be assessed in different ways, such as (i) Phenotypic parameters, which are characterized in 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 usedfor estimation the degree of genetic divergence is(IV) molecular techniques. The most important of molecular 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 of2-6 b.p. of repeated sequences (Chambers & MacAvoy, 2000; Hamdalla, 2009, and Smith et al., 1997) or 1-10 b.p. (Bruford & Wayne, 1993) such as (TG) n or (AAT) n. These parameters are spread widely in the genome of eukaryotic organisms (humans, animals and plants) and are frequently high covariance (Jaroslava et al., 2002).SSR markers assist in the classification of genotypes through identification 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 (Nandi et al., 1997), while the doubling of DNA is responsible for the differences among them (Olsen et al., 1993). Microsatellite is characterized as having high polymorphism, has capacity to produce varied bonds significantly compared to other markers and co-dominance. The most important uses of SSR is to estimate the diversity , genetic mapping and fingerprinting (Hao et al., 2006 , Huang et al., 2002), also it could provide a model for estimation thevariation among isolated generations (Manjarrez-Sandoval et al., 1997) and assists in improvement of crops efficiently through diagnosis the favorite alleles and collecting them. SSR markershaveemployed by many researchers in the genetic diversity studies of the various crops, mainly wheat (Akkaya & Buyukunal-Bal, 2004; Cadalen et al., 1997; Chao et al., 1989; Dje et al., 2000; Dograr et al., 2000; Kam-Morgan et al., 1989; Li et al., 2000; , Parasad et al., 2000; Pillen et al., 2000; , Singh et al., 2008; , Singh et al., 2010; , Sonja et al., 2012; and

Wange et al., 2006).Many studies have shown that SSR alleles located in the A and B genome compared to D (Röder et al., 1998 and Stephenson et al., 1998) .The disagreement over the distribution of these alleles across the genome may effects in the classification of the genotypes (Parasad et al., 2000). The study aims to (1) determinate the efficient primer (s) in the allelic diagnosis (2) estimate the degree of diversity among genotypes studied, and (3) determinate the appropriate genotype that possess high degree of diversity to improve its traits through plant breeding programs.

## II. Materials & Methods

### 2.1Plant material

Fourteen genotypes of wheat bread selected for this study which has been entered from USA & Mexico with two Iraqi genotypes (M = Latifya and N = Ibah95) (table 1), with ten primers (table 2) to estimate the degree of divergence by using SSR marker. The current study has conducted at the department of field crops and horticultural / University ofNebraska during 2013.

**Table 1:** 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 |
| **BW49475** | I | Mexico | WAXWING*2/HEILO |
| **BW49286** | J | Mexico | UP2338*2/KKTS*2//YANAC |
| **BW49082** | K | Mexico | ROLF07*2/5/FCT/3/GOV/AZ//MUS/4/DOVE/BUC |
| **BW49398** | 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) |
| **IBAH95** | 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 |

### 2.2 DNA Isolated

The seeds have sown in pots under laboratory conditions (15-20$C^o$). The leaflets have been collected for each genotype at stage of two leaflets and kept in sterile tubes, thereafter have been crushed by a Tissue Lyser machine for five minutes. DNA was extracted according to Shaha and others (2009).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.

**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 |

**2.3 Polymerase chain Reaction (PCR)**

PCR reaction was carried out using a thermal cycler and reaction mixture (volume 25 µl) consist 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 temperature and period for each step of PCR reaction.PCR products were separated at the electrophoresis for 120 minutes on polyacrylamide gel, which was prepared 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 been examined by UV short wave in a UV pro-transiluminator and have photographed by a unit Imager Gel Documents.

**2.4 Data scoring and data analysis**

For the statistical analysis, the formed bonds per primer were considered as an allele 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 (1980):

PIC=1-∑ Pij2

Where P: is the repeat of allele through all the genotypes, while the degree of genetic divergence has estimated according to Nei and Li (1979).

**Table 3**: PCR program

| Step | Temperature (C̊ ) | Period(minutes) |
|---|---|---|
| **1** | 94 | 3 |
| **2** | 94 | 0.45 |
| **3** | 55 | 1 |
| **4** | 72 | 1 |
| **5** | Repeated step 2 for 34 times, then go to step 6 | - |
| **6** | 72 | |
| **7** | 4 | 4 |

## III. Results&Discussion

All the primers have producedvariant 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 IBAH95respectively) to 7 allele sites for wmc153 and wmc264 (appeared in all genotypes except for BW3-9830,BW3-9872,BW3-9832,CW15-6723andBW49398).Genotype BW49475 has had a highest number of alleles reached to 11 alleles across all the primers except xgwm132, gmw32 and gmw391, while genotype BW49082has 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 for genotypesBW3-9832 ,BW49398 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 performance across 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 BW3-9832 genotypes to 1.0 for number of genotypes at a rate of 0.80 (Table 4).The local genotype LATIFYAshowed high rate of genetic diversity (0.95) while local genotype,IBAH95,showed a moderate rate of genetic diversity (0.75). This refers to high probability of improving genotype LATIFYAas compared with genotype IBAH95by introducing genes from the studied genotypes, therefore the highest degree of genetic diversity of genotype LATIFYAwas happened with most of the studied genotypes, while the same degree of genetic diversity was happened between genotype IBAH95and only two genotypes only.

**Table 4:** Values of genetic diversity for the genotypes studied

| H | G | F | E | D | C | B | A | |
|---|---|---|---|---|---|---|---|---|
| 0.83 | 0.84 | 0.81 | 0.80 | 0.81 | 0.2 | 0.4 | - | **A** |
| 0.8 | 0.81 | 0.77 | 0.75 | 0.77 | 0.25 | - | | **B** |
| 0.8 | 0.81 | 0.77 | 0.75 | 0.77 | - | | | **C** |
| 0.81 | 0.83 | 0.4 | 0.77 | - | | | | **D** |
| 0.8 | 0.81 | 0.55 | - | | | | | **E** |
| 0.81 | 0.83 | - | | | | | | **F** |
| 0.38 | - | | | | | | | **G** |
| - | | | | | | | | **H** |

Figure 1 shows the results of cluster analysis, which has classified the genotypes into two groups depending on the basis of their performance 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, BW49398and LATIFYA, while Group II included BW3-9852 , CW15-6723, CW15-6732, BW49475, IBAH95, GOLDEN 86 and KLASIC.

**Table 4:** continued

| P | O | N | M | L | K | J | I | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.66 | 0.69 | 1 | 1 | 0.77 | 0.8 | 0.88 | **A** |
| 1 | 0.8 | 0.63 | 1 | 1 | 0.71 | 0.75 | 0.86 | **B** |
| 1 | 0.6 | 0.63 | 1 | 1 | 0.71 | 0.75 | 0.86 | **C** |
| 69 | 0.63 | 0.5 | 1 | 1 | 0.75 | 0.77 | 0.62 | **D** |
| 0.5 | 0.8 | 0.81 | 1 | 1 | 0.71 | 0.75 | 0.73 | **E** |
| 0.53 | 0.45 | 0.5 | 1 | 1 | 0.75 | 0.77 | 0.75 | **F** |
| 1 | 0.84 | 0.85 | 1 | 1 | 0.8 | 0.81 | 0.88 | **G** |
| 1 | 0.83 | 0.84 | 1 | 1 | 0.77 | 0.8 | 0.88 | **H** |
| 0.89 | 0.88 | 0.66 | 1 | 1 | 0.85 | 0.73 | - | **I** |
| 1 | 0.8 | 0.81 | 1 | 1 | 0.42 | - | | **J** |
| 1 | 0.77 | 0.8 | 0.75 | 1 | - | | | **K** |
| 1 | 1 | 1 | 0.55 | - | | | | **L** |
| 1 | 1 | 1 | - | | | | | **M** |
| 0.86 | 0.69 | - | | | | | | **N** |
| 0.57 | - | | | | | | | **O** |
| - | | | | | | | | **P** |

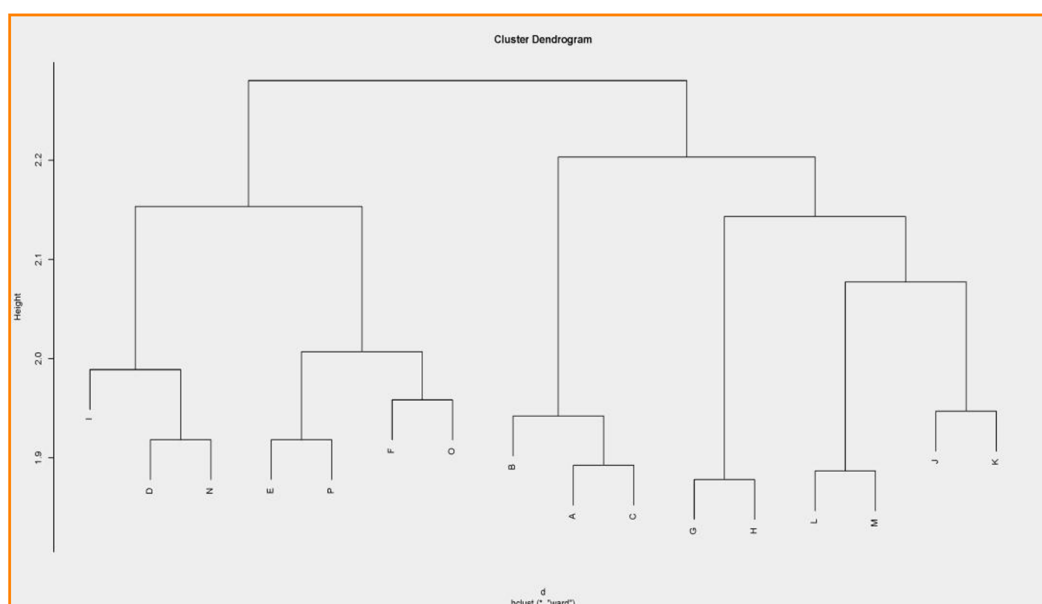


**Figure1:** The Cluster Analysis of the wheat genotypes based on them performances through Ten Primers

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 obtained PIC values in this study were high as compared to the values from previous studies, taking into account the number of studied genotypes. The values of diversity coefficient ranged between 0.2 for BW3-9830, BW3-9832 genotypes to 1.0 for numbers of genotypes at rate of 0.80. This 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 give another degree of divergence, hence use of wide range of primers is necessary for screening them and define their efficiency. 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 genotype BW49082 and in spite of its owning lowest number of alleles, which were diagnosed using the studied primers, it did not own highest rate of the genetic diversity value. This is because genotype BW49082 has identified the same alleles that have been identified by a number of the studied genotypes. We can conclude that the number of diagnosed alleles is not most important factor in estimation the degree of genetic diversity. Note that genotypes LATIFYA and IBAH95 have been located in two different groups although they are local, this because of difference in their genetic origins. Also notes that genotypes CW15-

6723 and CW15-6732, who they share same genetic origin, have located in Group II with the genotypes GOLDEN 86 and KLASIC, which they have been entered from north America. The same thing happened with the genotypes BW3-9872 and BW3-9832, who share the same genetic origin.Genotypes GOLDEN 86,KLASIC and IBAH95 were in one group while LATIFYA was in another group, therefore genotypes GOLDEN 86 and KLASIC, which have been entered from the United States, showed a complete degree of diversity (1.0) with genotype LATIFYA and less so with genotype IBAH95 (Figure 1).

**Table 5:** 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 |

## IV. Conclusion

The obtained high variance in this study refers to the possibility of utilizing these genotypes to improve the characters of the local genotypes through hybridization and selection within the breeding programs. Despite of the differences in frequency of SSR repeat parameters among the studied genotypes, there is high allelic rate; it is also showed highly divergence across wheat genome. The current study showed that the primers xwm132 and gmw32 possess high efficiency in detection of SSR alleles and can be used successfully in classification wheat genotypes in the subsequent studies. Also, the results of the study clearly indicated the possibility of using the studied genotypes to improve the characters of the local genotypes, especially genotype LATIFYA, which showed high degree of genetic divergence, bringing high degree of probability to receive new genes. Despite BW49398 genotype had highest rate of divergence degree, but it has not a capability to improve the genotype LATIFYAbecause they were part of the same group. It can be proposed to improve the local genotypes by hybridization with genotypes for other groups (hybridization local genotype, LATIFYA, with genotype(s) of group II and hybridization genotype IBAH95 with genotype(s) of Group I). As long as wheat crop is self-pollination, the variations within each genotype comes from genetic mutations, genetic drift, and then selection, so the heterogeneity of plants per genotype must be watched through genetic testing in order to prevent increasing heterogeneity to high levels.There is a difficulty in classification the wheat genotypes based on single marker due to complexity of the genome and great diversity among them, so using of different markers would provide a great tool for test the varieties.

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