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Genetic variation of ten Iraqi wheat genotypes using (SSR) markers and morphological characterization

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Abstract

The current study based on using morphological traits and simple sequence repeat(SSRs) markers to study variation among ten Iraqi wheat genotypes. Primers wmc596 and wmc603 produced three alleles distributed between one in wmc596 and two in wmc603 with an average number of 1.50 allele per locus . Primer wmc603 was more informative than wmc596 as produced PIC reached 0.3750. Morphological traits including whole plant (dry weight , height ,leaf number ,leaf area and branches number) , spike(dry weight ,length and number) and weight of 100 grain used for cluster analysis .Cluster analysis depending on morphological traits grouped wheat genotypes among two major groups , the first included only Faris genotype while the other large one included the rest genotypes which further divided in to two sub clusters. Genotypes identification and studying genetic variation produce an efficient tool for genotypes selecting in breeding programs.

Key word : Simple Sequence Repeat , Morphological traits , Genetic variation

Introduction:

Wheat considered an annual crop belongs to family Poaceae , this family contains number of most important species (Pathak and Shrivastav, 2015) , its one of high nutritional cereal crop (Shewry, 2007) .

Agronomically and nutrionally, wheat consider very important among other cereal crops because of its large size genome and wide range of uses (Motawei et al., 2007), thus its mainly for wheat improvement to determine local germplasm , germplasm diversity and genetic relationships among breeding lines (Abbas et al., 2008).

Genetic diversity studies is beneficial for plant breeding program since it can produce efficiently more plant species under diverse environmental conditions.(Khodadadi *et al*., 2011) .

Morphological characters are useful for wheat breeders to build crosses plan for positive traits. (Salem *et al* .,2008) as salinity tolerance genotypes (Ahmad *et al* .,2013).

Molecular markers could be used for examination of genotypes by tracing a desired gene(s). Aprotein or a piece of DNA can be used as a marker(Staub, *et al.*, 1996). DNA markers are considered best candidates for evaluation and selection of plant material efficiently. (Kumar *et al*., 2009).

Microsatellites ,knowning also as Simple Sequence Repeats (SSRs) which are parts of DNA,composing of tandemlyrepeats varying as mono-, di-, tri-,tetra- ,penta- up to six nucleotide units and organized throughout most genomes of eukaryote species (Powell *et al*. 1996a; Weising et al.,2005 and Kumar *et al*.,2009). Combination of morphological traits and SSR markers was valuable for studying germplasm variation including maize (Al-Badeiry,2013 ; Nikkhoy and Shiri, 2017and Gazal *et al*.,2018)), tomato(AL-Tamimi,2014) and wheat (Salem *et al*.,2008; Ahmad *et al*., 2013; Hassan,2016).

Material and methods

Sowing of grain seed of ten wheat genotypes Furat , Baghdad , Hashimia , Buhuth22, Latifia , Dijla, Abaa 99, Rasheed, Faris and Iraq was conducted at Adminstration of Agriculture in Najaf ,Coated Agriculture Project. Morphological traits including (leaf number ,plant height ,leaf area ,branches number and whole plant dry weight) and at maturity (weight of 100 seed grain ,spike number ,spike length and spike dry weight) then data entered to PAST statistic vital program, Version 62.1 (Hammer *et al.*, 2001) ,dendrogram was constructed based on genetic distance (GD=1-GS) using the Un weighted Pair-Group Method with Arithmetical Average (UPGMA).

For molecular variation study , fresh leaves were used for genomic DNA extraction using Genomic DNA Mini Kit at concentration of 76.91µg/ml with purity 1.9 followed by application of two simple sequence repeats (Wmc596: F-TCAGCAACAAACATGCTCGG R-CCCGTGTAGGCGGTAGCTCTT) and (Wmc603: F-ACAAACGGTGACAATGCAAGGA R-CGCCTCTCTCGTAAGCCTCAAC) amplified by polymerase chain reaction programmed as 3 min initial denaturation at 94°C, followed by 35 cycles ,each consisting of: 1 min at 94°C, 1 min at 61°C, 2min at 72°C and a final extension of 10 min at 72°C.(El-Assal and Gaber,2012**)** , amplified fragment separated using agarose gel at concentration of 2% , 70 volt for two hours , photographed and analyzed using Power Marker V 3.25 software (Liu and Muse, 2005).

Results and discussion

Constructed phyllogenetic tree depending on morphological traits in figure (1) indicate that wheat genotypes distributed among two major clusters , the first included only Faris while the other included nine genotypes and further can divided in to two sub clusters , the first small one included only Dijla while the other large one included (Furat , Baghdad , Hashimia , Buhuth22, Latifia , Abaa 99, Rasheed, and Iraq) genotypes .

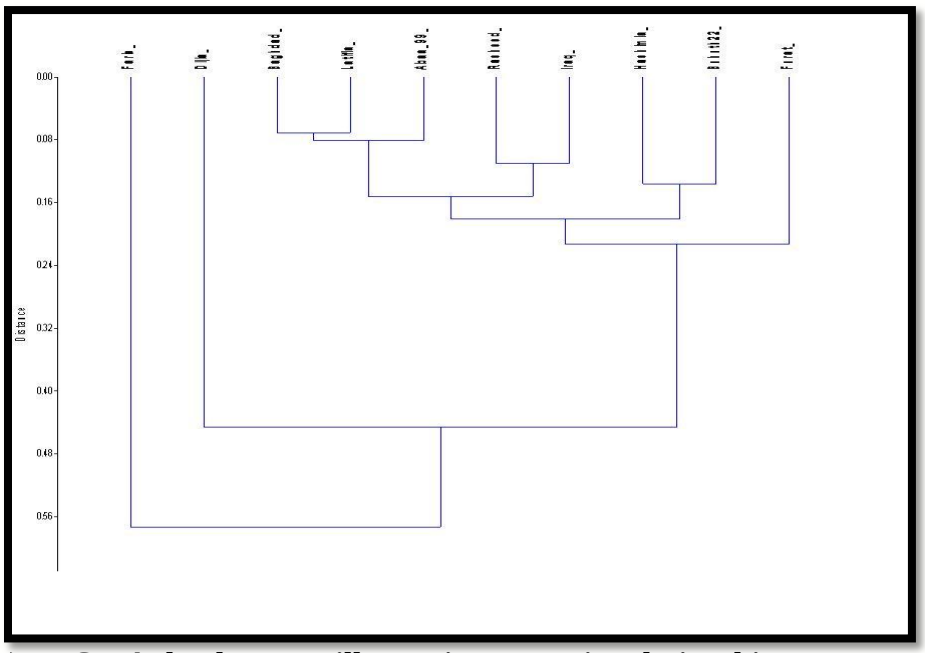

Figure (1) UPGMA dendrogram illustrating genetic relationship tree among wheat genotypes Furat , Baghdad , Hashimia , Buhuth22, Latifia , Dijla, Abaa 99, Rasheed, Faris and Iraq using morphological traits.

Data in table 1 and table 2 illustrate that primer wmc603 proceed primer wmc596 in producing two alleles at molecular size of 212bp and 126bp and other studded parameters , in addition to that PIC value reached 0.3750 which indicates that its more informative than **wmc596 (**Botstein *et al.* 1980). Figure 2 show agarose gel electrophoresis of amplification product of these two markers

Table 1: Data of SSR Markers including: Alleles size range bp , major allele frequency ,alleles number, ,gen diversity, Heterozygosity and polymorphic information content (PIC)

Locus	Molecular size in bp	Major.Allele. Frquency	Allele No.	Gene Diversity	Heterozygosity	PIC
wmc596	165	1.0000	1.0000	0.0000	0.0000	0.0000
wmc603	212-126	0.5000	2.0000	0.5000	1.0000	0.3750
Mean	-	0.7500	1.5000	0.2500	0.5000	0.1875

Table 2: SSR amplification product with primers wmc603 and wmc596 , molecular size (M. S) in base pair (bp) for ten wheat genotypes .

Locus	Wheat genotype									
	Furat	Baghdad	Hashimia	Buhuth22	Latifia	Dijla	Abaa 99	Rasheed	Faris	Iraq
wmc603	126	+	+	+	+	+	+	+	+	+
	212bp	+	+	+	+	+	+	+	+	+
wmc596	165bp	+	+	+	+	+	+	+	+	+

Results in table 3 show variation in all studied morphological traits among genotypes, plant height is genetically controlled trait ,but influenced by environmental conditions and management practices. The variation in plant height among diverse genotypes confirmed by Ram *et al*., (2012) and Al-Badeiry ,(2013) .

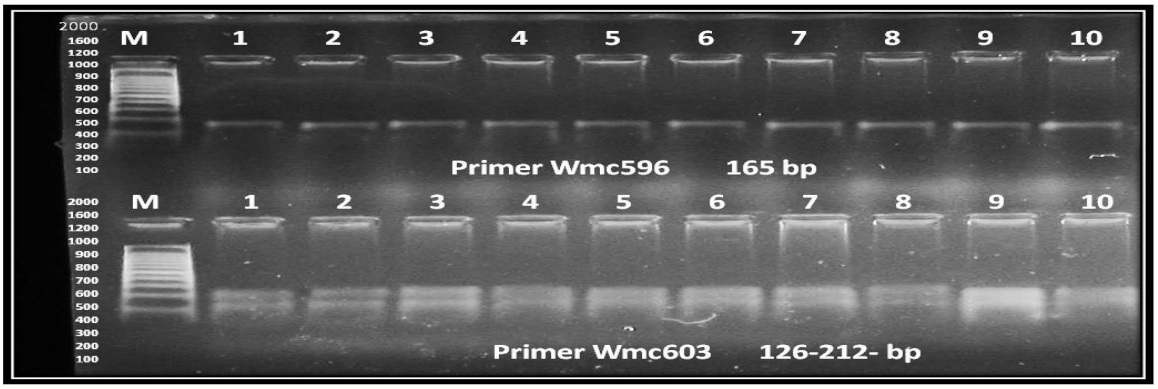

Figure2 SSR amplification product with primers wmc596 and wmc603 , lane M: DNA ladder and lanes1- Furat 2-Baghdad 3- Hashimia 4-Buhuth225-Latifia 6- Dijla7-Abaa 998-Rasheed, 9-Faris 10 -Iraq represent wheat genotypes.

Table 3 : Morphological characteristics of wheat genotypes .

Genotype name	Dry weight (gm)	Plant height (cm)	Leaf number	Leaf area ($cm^{2)}$	Branches number	Spike dry weight (gm)	Spike length (cm)	Spike number	Weight of 100 grain seed (gm)
Furat	4.0246	65.55	17.7	49.207	3.6	28.01632	32.81466	26.2676	27.98112
Baghdad	7.6849	73.5	20.8	52.43	4.5	31.78298	36.6026	29.22312	30.90774
Hashimia	3.918	64.9	25.9	50.925	5.5	30.2286	35.49072	29.60886	30.35064
Buhuth22	11.3918	70.4	24.8	51.445	5.8	32.76736	37.04247	30.37097	31.48516
Latifia	4.2911	76.05	21.4	54.858	4.5	32.21982	37.80556	30.15668	31.90801
Dijla	18.0601	78.45	46.8	47.245	5.3	39.17102	43.3932	36.38184	34.29821
Abaa 99	4.8601	78.6	20.7	49.078	4.3	31.50762	36.83712	28.48455	30.04146
Rasheed	8.7293	68.96	21.5	56.324	3.1	31.72266	36.32133	29.7936	31.45232
Faris	2.6109	54.35	15.9	31.4	3	21.45218	25.22044	19.39452	20.09343
Iraq	5	66.95	17.8	62.67	3.6	31.204	36.4448	30.34376	32.85251

A linkage of genetics and morphological traits could in future help plant breeders in diagnosis traits closely linked to productivity ,in general, morphological characterization provide a very simple technique of quantifying genetic variation (Fufa *et al*., 2005; Shuaib *et al*., 2007and Al-Badeiry ,(2013)). Low variation revealed by SSR two markers doesn't match variation revealed by morphological traits ,this established by Salem *et al* .,(2008).

DNA markers and morphological traits not necessary matching closely results (Vollmann *et al*.,2005 and Martnez *et al*.,2005). Two reasons for this low correlation between DNA markers and morphological traits in addition to protein data: 1- DNA markers covering most of genome region , including coding and non coding regions 2- DNA markers are less subjected to artificial selection in comparing to morphological traits(Semagn .,2002). Increasing number of markers , traits and genotypes number in analyzing genome diversity is essential to estimates parent selection and gain maximum value and practical impact in future breeding program.

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