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Genetic analysis of ten cultivars of bread wheat in Iraq Using microsatellite(SSR) Markers

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ABSTRACT

The study was carried out to estimate genetic variability of ten Iraqi wheat varieties by using microsatellite . PCR amplifications with 15 SSR markers gave 266 loci of which 248 (93.2%) polymorphic with PIC value 0.4308. The pattern generated by SSR markers separated the ten wheat varieties into two clusters. Dendrogram of similarity by the (UPGMA). Study appered two groups including a group closest genetically Tamouz3, Ibaa99, Tamouz2 in same group while the other cultivars distributed according to the genetic distance and the results showed the two cultivars Rasheed and Sham4 are the most distant genetically.

microsatellite has a best markers than another genetic techniques for identify genetic diversty between cultivars, and known the descent of relatives, and. ancestors. It is an ideal marker that assessing the genetic diversity in population of wheat.

التحليل الوراثي لعشرة اصناف من القمح في العراق باستخدام مؤشرات المايكروستلايت(SSR)

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الخلاصة

أجريت هذه الدراسة لتقدير التباين الوراثي لعشرة أصناف من حنطة الخبز العراقي باستخدام تقنية المايكروستلايت (SSR) أعطى تضخيم التسلسل التكراري مع ١٥ بادئ 266 موضعًا، منها ٢٤٨ (٩٣.٢%) متعددة الأشكال بقيمة PIC 0.4308. اظهر الطراز الناتج عن مؤشرات الـ SSR بفصل أصناف القمح العشرة إلى مجموعتين. تم الحصول على Dendrogram ومصفوفة التشابه من خلال تحليل طريقة المجموعة الزوجية غير المرجحة (UPGMA). أظهرت الدراسة مجموعتين إحداهما المجموعة الأقرب وراثياً

تموز٣، إباء٩٩، تموز٢ في نفس المجموعة بينما توزعت الأصناف الأخرى حسب المسافة الوراثية وأظهرت النتائج أن الصنفين رشيد وشام٤ هما الأكثر بعداً وراثياً.
يمتلك SSR علامة جزيئية أفضل من التقنيات الجزيئية الأخرى للكشف عن التنوع الوراثي بين الأصناف، والمساعدة في معرفة نسب الأقارب والاسلاف وهو علامة مثالية لتقييم التنوع الوراثي في اصناف القمح.
الكلمات المفتاحية: علامات SSR وشجرة المسافة الوراثية والتنوع الاليلي

INTRODUCTION

Bread wheat (2n = 6x = 42) belongs to Poaceae, family. (Łaba et al,2022) It product major eaten grains and supply about one-half of humans' food calories and a major part of their nutritive demandment . In the last three year the universal wheat acreage was 213.9–219.0 million and yields from 732.1 to 760.9 million tonnes (FAOSTAT,2022).

As one of the fundamental grain harvest, wheat provides 20% of the calories and proteins used by humans. Due to population amplification, dietary transfer and climate alteration, it is challenging for wheat breeder to develop new varities of wheat to endure biotic ansd a abiotic stress (Erenstein et al, 2022) and (Min et al, 2012).

wheat is a important cereal crop overripe under various climatic situation in various parts of the world. Different physiological mechanisms, stay-green, high resiperation rate, and photosynthetic rates are utilize by the plants to conform to excessive temperature stress. Under late happening or another heading of high temperature stress, earliness supply an escape mechanism .

Genetic doctrinaire is the better path to raise up wheat production , thus , necessary to assessment genotypes depend on genetic diversity and genetic parameters in wheat breeding programs. The decreasing of wheat productivity in different countries because of shortage of improved varities. various of genetic markers have been used to study of genetic diversity, genetic characterization, genome mapping (Liqiang et al,2023), (Iqbal et al, 2020), (Joshi et al, 2007), (Kumari et al, 2019), (Tyagi et al, 2021) and (Tyagi et al, 2019).

However, among these different types of markers, microsatellite are deemed as markers of choice due to their various advantages as parallel to other marker (Gupta et al, 2013), (Kumar et al, 2019), and (Mir andVarshney,2013) microsatellite, among of genetic markers, are used to the genetic diversity analysis, QTL mapping, and marker-assisted breeding (Mir et al, 2013) and (Munusamy et al, 2017). The upon a time studies proposed that the microsatellite present during the genes are more suitable for genetic breeding than random genomic microsatellite most of which are obtain from the protein-coding or UTRsr egions of the plant genomes (Abouzied et al, 2013). The markers are used to identity alleles controlled on the traits in the genotypes of plants (Ferrari,2007).

Thus , it was insistent to morphological and genetic characterization wheat genotypes for soon ripeness and to validate then specified markers associated with soon ripeness. Therefore, The aim of this study was conducted to estimated the genetic diversity by using identified marker-trait associations (MTAs) to maturity and estimate genetic parameters in conventional palnt breeding methods (Liqiang et al,2023).

MATERIALS AND METHODS

DNA extraction

DNA genomic was extracted using the CTAB method Ferrari et al (Ciuca and Petcu,2009). evaluated of DNA concentration quality was performed and running on 0.8% agarose gels. .

Genetic Analysis

Fifty wheat SSR primer pairs Performing all wheat genomic were elected and used for screening wheat genotypes (Table 1). Sequences of SSR markers and PCR conditions were obtained by Grain Genes Database (http://wheat.pw.usda. gov).

PCRs were performed according to Ciucă and Petcu (Doveri,2006) in a 10 ,µL volume consist of 30 ng, of DNA, 0.5, unit of Fast -Start Taq Polymerase, 1 × buffer , 2.5 mM MgCl2, 200 µM of each dNTP (Invitrogen, Brazil), 0.1 µM of tailed forward. primer, 0.5 Mm tailed labeled with IRD700 fluorophore 0.5 µM of reverse primer ,The forward primer was "tailed" by the implying of 19 nucleotides at the 5' end, which expedite of the products, reactions were carried out in a thermo cycler with the following profile: 95 °C for 5min, 6 cycles at 95 °C for 20 s, annealing temperature for 30 s, decreasing 1 °C/cycle, extension temperature 72 °C for 30 s; put up with 29 cycles at 95 °C for 20 s, annealing temperature 50 °C for 30 s, 72 °C for 30 s with a final extension at 72 °C for 6 min. (Table 2) . primer were profiled using a 4300 DNA sequencing , 1 µL of the product was loaded onto a 6% polyacrylamide gel, with Marker standards and electrophoreses at 1500 V. (Roldan-Ruiz,2000).

A 100bp DNA Ladder was used to respect the size of amplified DNA fragments. delusive polymorphisms were exposed for each marker. Percentage of the polymorphism obtained by each microsatellite was calculated. to inspected the expediency of each marker to estimate the genetic variety among the wheat studied, polymorphism information content (PIC) following(Powell et al, 1996) and marker index (MI) using the method of (Nei and Li,1979) was also calculated**.**

Table 1 microsatellite sequence for (15) loci with labeled tail (CAC GAC GTT GTA AAA CGAC) used in the amplification of wheat .

primer	Forward	Reverse	(An. Temp)	Opata (bp)	Synth. (bp)
Xgwm111-7D	5`-TCT GTA GGC TCT CTC CGA CTG-`3	5`-ACC TGA TCA GAT CCC ACT CG-`3	55º	206	184
Xgwm120-2B	5`-GAT CCA CCT TCC TCT CTC TC-`3	5`-GAT TAT ACT GGT GCC GAA AC-`3	60º	162	174
Xgwm162-3A	5`-AGT GGA TCG ACA AGG CTC TG-`3	5`-AGA AGA AGC AAA GCC TTC CC-`3	60º	202	208
Xgwm174-5D	5`-GGG TTC CTA TCT GGT AAA TCC C-`3	5`-GAC ACA CAT GTT CCT GCC AC-`3	55º	233	204
Xgwm264-1B	5`-GAG AAA CAT GCC GAA CAA CA-`3	5`-GCA TGC ATG AGA ATA GGA ACT G-`3	60º	157	165
Xgwm299-3B	5`-ACT ACT TAG GCC TCC CGC C-`3	5`-TGA CCC ACT TGC AAT TCA TC-`3	55º	206	215
Xgwm408-5B	5`-TCG ATT TAT TTG GGC CAC TG-`3	5`-GTA TAA TTC GTT CAC AGC ACG C-`3	55º	182	148
Xgwm448-2A	5`-AAA CCA TAT TGG GAG GAA AGG-`3	5`-CAC ATG GCA TCA CAT TTG TG-`3	60º	203	243
Xgwm459-6A	5`-ATG GAG TGG TCA CAC TTT GAA-`3	5`-AGC TTC TCT GAC CAA CTT CTC G-`3	55º	118	126
Xgwm577-7B	5`-ATG GCA TAA TTT GGT GAA ATT G-`3	5`-TGT TTC AAG CCC AAC TTC TAT T-`3	55º	164	155
Xgwm608-4D	5`-ACA TTG TGT GTG CGG CC-`3	5`-GAT CCC TCT CCG CTA GAA GC-`3	60º	151	144
Xgwm635-7D	5`-TTC CTC ACT GTA AGG GCG TT-`3	5`-CAG CCT TAG CCT TGG CG-`3	60º	99	93
Xgwm639-5B	5`-CTC TCT CCA TTC GGT TTT CC-`3	5`-CAT GCC CCC CTT TTC TG-`3	55º	166	170
Xgwm46-7B	5`-GCA CGT GAA TGG ATT GGA C-`3	5`-TGA CCC AAT AGT GGT GGT CA-`3	60º	186	179
Xgwm95-2A	5`-GAT CAA ACA CAC ACC CCT CC-`3	5`-AAT GCA AAG TGA AAA ACC CG-`3	60º	128	116

Investigation of data

Just reproducible and clear cut bands in the replications were considered as expected polymorphic markers, for every primer, the bands were scored as 1 (present) or 0 (missing), and hereditary comparability was assessed utilizing Nei-Li's similitude file (Mohammadi and Prasanna,2023). A dendrogram was built based on the closeness network information by UPGMA

RESULTS

SSR analysis of ten cultivar of bread wheat utilizing fifteen microsatellite loci gave a sum of 266 bands as show in Table 2. 248 of these loci (93.2%) were polymorphic over every one of the genotypes investigation. The typical number of uncovered alleles per locus was 3.93 while the typical number of distinguished bands per loci was 15.93.

The allele numbers and allele sies of the markers are gifted in Table 2. The number of alleles detected by the markers ranged from 1 to 5 among the wheat genotypes. The most polymorphic SSR marker was Xgwm577-7B with 5 alleles, followed by Xgwm635-7D, and Xgwm639-5B they all had 4 alleles . A total 266 polymorphic allele were gained from screening 10 wheat cultivars using the 15 microsatellite markers with an average of 2.6 alleles per locus. PIC values ranged from 0.000 (Xgwm608-4D.) to 0.703 (Xgwm639-5B) with an average of 0.4308 for selected primers.

The lowest number of alleles per locus and the PIC was calculated to be 1 and 0.000 respectively in Xgwm608-4D. Loci Xgwm577-7B, Xgwm635-7D and Xgwm639-5B were appropriate for mapping the genomic, while the other concentrated on loci were missionary markers for wheat and accord with the result revealed in investigation of Tunisian wheat cultivars (Carvalho et al ,2008). The largest number of polymorphic bands over all assortments distinguished by loci Xgwm639-5B this distinction between loci bands yield in view of the changed of. The, markers .

Table 2. SSR analysis of ten cultivated

primers	Polymorphic Information Content	Marker efficacy	bands	Alleles	(bp) of Alleles	Mono morphic	%	Polym orphic	%
Xgwm111-7D	0.359	Informative marker	17	2	135,143	1	5.8	16	94.2
Xgwm120-2B	0.374	Informative marker	19	2	230.235	1	5.2	18	94.8
Xgwm162-3A	0.437	Informative marker	17	3	218,220, 232	1	5.8	16	94.2
Xgwm174-5D	0.375	Informative marker	14	2	145,174	0	0.00	14	100.00
Xgwm264-1B	0.372	Informative marker	18	2	152,180	1	5.5	17	94.5
Xgwm299-3B	0.374	Informative marker	15	2	177,180	0	0.00	15	100.00
Xgwm408-5B	0.346	Informative marker	15	2	188,190	1	6.6	14	95.4
Xgwm448-2A	0.571	Informative marker	23	3	170,173,180	0	0.00	23	100.00
Xgwm459-6A	0.346	Informative marker	12	2	140,155	0	0.00	12	100.00

Xgwm577-7B	0.587	Suitable for mapping	14	5	147,175,180,200,228	0	0.00	14	100.00
Xgwm608-4D	0.000	Informative marker	10	1	147	10	100.00	0	0.00
Xgwm635-7D	0.697	Suitable for mapping	29	4	180,182,210,212	0	0.00	29	100.00
Xgwm639-5B	0.703	Suitable for mapping	39	4	151,168,170,181	3	6.7	36	92.3
Xgwm46-7B	0.589	Informative marker	14	3	162,165,180	0	0.00	14	100.00
Xgwm95-2A	0.332	Informative marker	10	2	145,147	0	0.00	10	100.00
Total			266	39		18	7.6	248	93.2
Averg	0.4308								

Percent Disagreement Values were used to locate the genetic distancesdimension between the wheat cultivars. Where high values of this matrix display the genetic diversity, and when it increases the genetic diversity studied cultivars. For SSR primer , PDV values extended from 0.107 between Ibaa99 and each of Tamouz3 and Tamouz2 which show a high degree of genetic similarity, to 0.333 between Rasheed and Sham 4 which is show a high degree of genetic variation. (Table 3).

Table 3 PDV produced by 15 SSR markers

OTU	Sham4	Nour	Latifieh	Tamouz3	Iraq	Ibaa99	Tamouz2	Abu-Ghareeb	Rasheed	Furat
Sham4	**0.000**									
Nour	**0.158**	**0.000**								
Latifieh	**0.222**	**0.228**	**0.000**							
Tamouz3	**0.185**	**0.193**	**0.185**	**0.000**						
Iraq	**0.207**	**0.285**	**0.132**	**0.245**	**0.000**					
Ibaa99	**0.143**	**0.152**	**0.25**	**0.107**	**0.237**	**0.000**				
Tamouz2	**0.222**	**0.228**	**0.296**	**0.148**	**0.283**	**0.107**	**0.000**			
Abu-Ghareeb	**0.28**	**0.170**	**0.32**	**0.28**	**0.265**	**0.192**	**0.24**	**0.000**		
Rasheed	**0.333**	**0.297**	**0.241**	**0.215**	**0.28**	**0.320**	**0.294**	**0.276**	**0.000**	
Furat	**0.207**	**0.25**	**0.207**	**0.170**	**0.153**	**0.2**	**0.245**	**0.224**	**0.12**	**0.000**

The studied bread wheat varieties were distributed into Two major categories (Figure 1). Two subgroups emerged from the first major categories. the initial one contained a Cultivars, Abu-Ghareeb, while the secondary categories was divided into two sub categories. The first comprisedSham4 and

Nour varieties, and the second included three varieties, namely Tamouz3 Ibaa99 and Tamouz2. The second major categories, in turn, was divided into two secondary categories, the first included Latifieh and Iraq varieties while the second included Rasheed, and Furat varieties The genetic tree showed that the two varieties closest to each other were Tamouz3 and Ibaa99, with a similarity percentage of 89%, while the two farthest varieties varieties from each other were Rasheed and Sham4, with a similarity percentage of 66%..This result could provide the potential of finding useful associations between these markers .

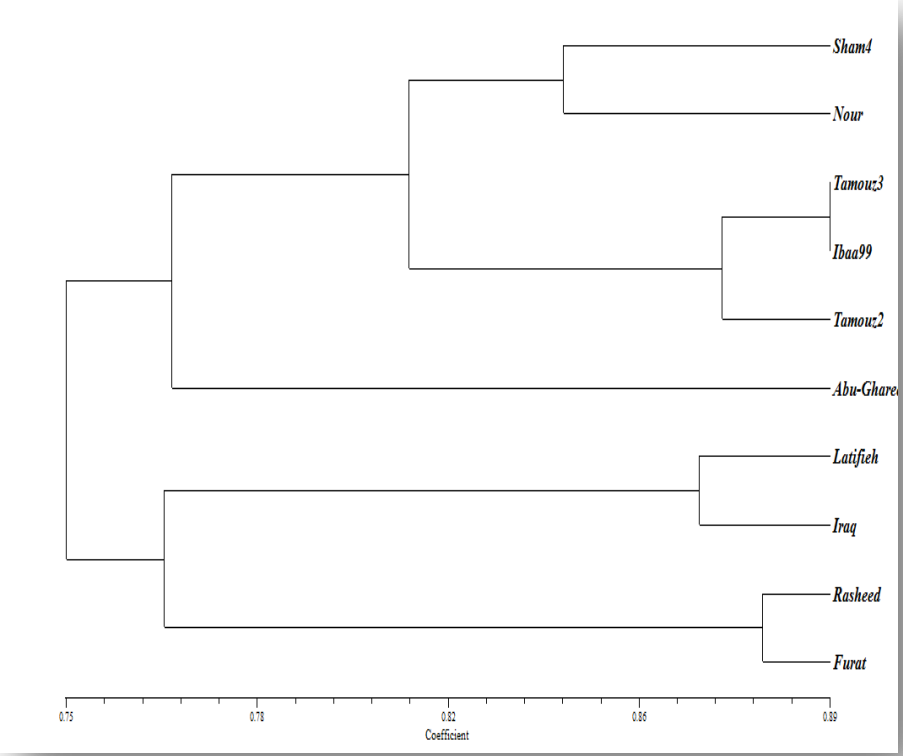

Fig. 1. cluster tree analysis of 10 bread wheat cultivar with 15 SSR primer.

CONCLUSIONS

Preserving germplasm and implementing breeding programs require an understanding of the degree of genetic variability among approximate populations. A respectable level of genetic variety was evident in the examined wheat accessions for the estimated quantitative features. The SSR primer we assessed in our study offered rising fingerprinting profiles and appropriate polymorphism for assessing genetic variation in wheat.

According to their genetic distance space, the study makes it easier to choose the parents for hybridization. It is suggested that the SSR primer system is useful for molecular diversity analysis in germplasm preservation and has been shown to be successful in genetically differentiating cultivars. likewise The substantial polymorphism rate among wheat variations demonstrated the effectiveness of the SSR approach in analyzing genetic diversity among wheat types.

We can conclude the using of SSR markers to discriminate between of wheat genotypes by microstalte ,because the analyazed of wheat accessions appears genetic diversiy more acurity compare with converntional plant breeding methods . the best gentic diversity between Rasheed and sham4, IBAA and Rasheed . finally , we can used Rasheed , Sham4 and Ibaa to improved wheat genotypes by depend hybrid vigor vis cross between of them.

REFERENCES

Abouzied HM, Eldemery SMM, Abdellatif KF (2013) SSRbased genetic diversity assessement in tetraploid and hexaploid wheat populations. British Biotechnol J 3:390–404

Ciucă, M.; Petcu, E. (2009). SSR markers associated with membrane stability in wheat (Triticum aestivum L.). Rom. Agric. Res., 26, 21–24.

Doveri, S.; O'Sullivan, D.M. and Lee, D. (2006) . Non-concordance between genetic profiles of olive oil and fruit: A cautionary note to the use of DNA markers for provenance testing, Journal of Agriculture and Food Chemistry 54 ;9221-9226.

Erenstein O, Jaleta M, Mottaleb KA, Sonder K, Donovan J, Braun H-J.(2022) Global trends in wheat production, consumption and trade. In: Reynolds MP, Braun HJ, editors. Wheat improvement: food security in a changing climate. Cham: Springer International Publishing2. pp. 47–66.

FAOSTAT. Available online: **https://www.fao.org/** (accessed on 29 November 2022).

Ferrari, C.d.S.; Valente, L.L.; Brod, F.C.A.; Tagliari, C.; Sant'Anna, E.S.; Arisi, A.C.M.(2007) Evaluation of polymerase chain reaction and DNA isolation protocols for detection of genetically modified soybean. Int. J. Food Sci. Technol. 42, 1249–1255.

Gupta PK, Rustgi S, Mir RR (2013) Array-based highthroughput dna markers and genotyping platforms for cereal genetics and genomics. In: Gupta PK, Varshney RK (eds) Cereal genomics II. Springer, The Netherlands.

Iqbal MS, Singh AK, Ansari MI.(2020). Effect of drought stress on crop production. In: Rakshit A, Singh H, Singh A, Singh U, Fraceto L (eds), New frontiers in stress management for durable agriculture, Springer, New York, U. S. A.

Joshi AK, Mishra B, Chatrath R, Ferrara GO, Singh RP (2007) Wheat improvement in India: present status emerging challenges and future prospects. Euphytica 157:431–446.

Kumar A, Sharma A, Gahlaut V, et al. (2019) Genome-wide identification, characterization, and expression profiling of SPX gene family in wheat. Int J Biol Macromol 140:17–32. pmid:31419556.

Kumari RR Mir, Tyagi S, Balyan HS, Gupta PK (2019) Validation of QTL for grain weight using MAS-derived pairs of NILs in bread wheat (Triticum aestivum L.). J Plant Biochem Biotech 28(3):336–344.

Łaba, S.; Cacak-Pietrzak, G.; Łaba, R.; Sułek, A.; Szczepański, K. Food Losses in Consumer Cereal Production in Poland in the Context of Food Security and Environmental Impact. *Agriculture* **2022**, *12*, 665.

Liqiang, S. Ruihui, W. Xueju ,Y. Aimin, Z and Dongcheng, Liu.(2023). Molecular Markers and Their Applications in Marker-Assisted Selection (MAS) in Bread Wheat (Triticum aestivum L.) Agriculture, 13, 642. https://doi.org/10.3390/agriculture13030642.

Min K, Na Kang Y, Kim C, Choi C, Kim A.(2021) Genetic diversity and population structure of korean common wheat (*Triticum aestivum*). Korean J Breed Sci.53:277–88.

Mir RR and Varshney RK (2013) Future prospects of molecular markers in plants. In: Henry Robert J. (eds.), Molecular Markers in Plants. Blackwell Publishing Ltd, Oxford, UK., pp. 169–190. ISBN 9781118473023.

Mir RR, Hiremath PJ, Riera-Lizarazu O, Varshney RK (2013) Evolving molecular marker technologies in plants: from RFLPs to GBS. In: Lu¨bberstedt Thomas and Varshney R.K (Eds.) Diagnostics in Plant Breeding. Springer, Dordrecht, pp 229–247.

Mohammadi. S.A.; Prasanna. B.M.(2003). Analysis of genetic diversity in crop plants: salient statistical tools and considerations cropscie . 43: 1235-1248.

Munusamy P, Zolotarov Y, Meteignier LV, Moffett P, Stro¨mvik MV (2017) De novo computational identification of stress-related sequence motifs and microRNA target sites in untranslated regions of a plant translatome. Sci Rep 7: 43861. https://doi.org/10.1038/srep43861 PMID: 28276452.

Nei, M. and W.H. Li.(1979). Mathematical model for studying genetic variations in terms of restriction endonucleases. Proc. Nat. Acad. Sci., 76: 5269-5273. 22.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, et al. (1996). The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for gemplasm analysis. Mol. Breed. 2: 225-238**.**

Roldan-Ruiz I, Dendauw J, Vanbockstaele E, Depicker A, De Loose M.(2000). AFLP markers reveal high polymorphic rates in ryegrasses (Lolium spp.). Mol. Breed. 6: 125-134.

Tyagi S, Kumar A, Gautam T, Pandey R, Rustgi S, Mir RR (2021) Development and use of miRNA-derived SSR markers for the study of genetic diversity population structure and characterization of genotypes for breeding heat tolerant wheat varieties. PLoS One 16(2):e0231063.

Tyagi S, Sharma S, Ganie SA, Tahir M, Mir RR, Pandey R (2019) Plant microRNAs: biogenesis, gene silencing, webbased analysis tools and their use as molecular markers. Biotech 9(11):413.

Carvalho, A., J.L. Brito, B. Macas, H.G. Pinto, 2008. Genetic variability analysis of a collection of Old Portuguese bread wheat using ISSRs. Options Mediterraneennes. Ser. A, Sem. Medit., 81:35-38. 4 ref.