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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.

### KEY WORDS:

SSR markers, Genetic distance dendrogram, Allelic diversity

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## 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 15 بادئ 266 موضعاً، منها 248 (93.2%) متعددة الأشكال بقيمة PIC 0.4308. اظهر الطراز الناتج عن مؤشرات ال- SSR بفصل أصناف القمح العشرة إلى مجموعتين. تم الحصول على Dendrogram ومصنفة التشابه من خلال تحليل طريقة المجموعة الزوجية غير المرجحة (UPGMA). أظهرت الدراسة مجموعتين إحداهما المجموعة الأقرب وراثياً تموز3، إباء99، تموز2 في نفس المجموعة بينما توزعت الأصناف الأخرى حسب المسافة الوراثية وأظهرت النتائج أن الصنفين رشيد وشام4 هما الأكثر بعداً وراثياً.

يمتلك SSR علامة جزيئية أفضل من التقنيات الجزيئية الأخرى للكشف عن التنوع الوراثي بين الأصناف، والمساعدة في معرفة نسب الأقارب والأسلاف وهو علامة مثالية لتقييم التنوع الوراثي في اصناف القمح.

## 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 varieties of wheat to endure biotic and 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 respiration 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 varieties. 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 MgCl<sub>2</sub>, 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) |
|----------------|--|--|------------|------------|-------------|
| Xgwm11<br>1-7D | 5'-TCT GTA GGC TCT CTC CGA CTG- <sup>3</sup>   | 5'-ACC TGA TCA GAT CCC ACT CG- <sup>3</sup>    | 55°        | 206        | 184         |
| Xgwm12<br>0-2B | 5'-GAT CCA CCT TCC TCT CTC TC- <sup>3</sup>    | 5'-GAT TAT ACT GGT GCC GAA AC- <sup>3</sup>    | 60°        | 162        | 174         |
| Xgwm16<br>2-3A | 5'-AGT GGA TCG ACA AGG CTC TG- <sup>3</sup>    | 5'-AGA AGA AGC AAA GCC TTC CC- <sup>3</sup>    | 60°        | 202        | 208         |
| Xgwm17<br>4-5D | 5'-GGG TTC CTA TCT GGT AAA TCC C- <sup>3</sup> | 5'-GAC ACA CAT GTT CCT GCC AC- <sup>3</sup>    | 55°        | 233        | 204         |
| Xgwm26<br>4-1B | 5'-GAG AAA CAT GCC GAA CAA CA- <sup>3</sup>    | 5'-GCA TGC ATG AGA ATA GGA ACT G- <sup>3</sup> | 60°        | 157        | 165         |
| Xgwm29<br>9-3B | 5'-ACT ACT TAG GCC TCC CGC C- <sup>3</sup>     | 5'-TGA CCC ACT TGC AAT TCA TC- <sup>3</sup>    | 55°        | 206        | 215         |
| Xgwm40<br>8-5B | 5'-TCG ATT TAT TTG GGC CAC TG- <sup>3</sup>    | 5'-GTA TAA TTC GTT CAC AGC ACG C- <sup>3</sup> | 55°        | 182        | 148         |
| Xgwm44<br>8-2A | 5'-AAA CCA TAT TGG GAG GAA AGG- <sup>3</sup>   | 5'-CAC ATG GCA TCA CAT TTG TG- <sup>3</sup>    | 60°        | 203        | 243         |
| Xgwm45<br>9-6A | 5'-ATG GAG TGG TCA CAC TTT GAA- <sup>3</sup>   | 5'-AGC TTC TCT GAC CAA CTT CTC G- <sup>3</sup> | 55°        | 118        | 126         |
| Xgwm57<br>7-7B | 5'-ATG GCA TAA TTT GGT GAA ATT G- <sup>3</sup> | 5'-TGT TTC AAG CCC AAC TTC TAT T- <sup>3</sup> | 55°        | 164        | 155         |
| Xgwm60<br>8-4D | 5'-ACA TTG TGT GTG CGG CC- <sup>3</sup>        | 5'-GAT CCC TCT CCG CTA GAA GC- <sup>3</sup>    | 60°        | 151        | 144         |
| Xgwm63<br>5-7D | 5'-TTC CTC ACT GTA AGG GCG TT- <sup>3</sup>    | 5'-CAG CCT TAG CCT TGG CG- <sup>3</sup>        | 60°        | 99         | 93          |
| Xgwm63<br>9-5B | 5'-CTC TCT CCA TTC GGT TTT CC- <sup>3</sup>    | 5'-CAT GCC CCC CTT TTC TG- <sup>3</sup>        | 55°        | 166        | 170         |
| Xgwm46-<br>7B  | 5'-GCA CGT GAA TGG ATT GGA C- <sup>3</sup>     | 5'-TGA CCC AAT AGT GGT GGT CA- <sup>3</sup>    | 60°        | 186        | 179         |
| Xgwm95-<br>2A  | 5'-GAT CAA ACA CAC ACC CCT CC- <sup>3</sup>    | 5'-AAT GCA AAG TGA AAA ACC CG- <sup>3</sup>    | 60°        | 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 distances dimension 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       | <b>0.000</b> |              |              |              |              |              |              |              |              |              |
| Nour        | <b>0.158</b> | <b>0.000</b> |              |              |              |              |              |              |              |              |
| Latifieh    | <b>0.222</b> | <b>0.228</b> | <b>0.000</b> |              |              |              |              |              |              |              |
| Tamouz3     | <b>0.185</b> | <b>0.193</b> | <b>0.185</b> | <b>0.000</b> |              |              |              |              |              |              |
| Iraq        | <b>0.207</b> | <b>0.285</b> | <b>0.132</b> | <b>0.245</b> | <b>0.000</b> |              |              |              |              |              |
| Ibaa99      | <b>0.143</b> | <b>0.152</b> | <b>0.25</b>  | <b>0.107</b> | <b>0.237</b> | <b>0.000</b> |              |              |              |              |
| Tamouz2     | <b>0.222</b> | <b>0.228</b> | <b>0.296</b> | <b>0.148</b> | <b>0.283</b> | <b>0.107</b> | <b>0.000</b> |              |              |              |
| Abu-Ghareeb | <b>0.28</b>  | <b>0.170</b> | <b>0.32</b>  | <b>0.28</b>  | <b>0.265</b> | <b>0.192</b> | <b>0.24</b>  | <b>0.000</b> |              |              |
| Rasheed     | <b>0.333</b> | <b>0.297</b> | <b>0.241</b> | <b>0.215</b> | <b>0.28</b>  | <b>0.320</b> | <b>0.294</b> | <b>0.276</b> | <b>0.000</b> |              |
| Furat       | <b>0.207</b> | <b>0.25</b>  | <b>0.207</b> | <b>0.170</b> | <b>0.153</b> | <b>0.2</b>   | <b>0.245</b> | <b>0.224</b> | <b>0.12</b>  | <b>0.000</b> |

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 comprised Sham4 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 .

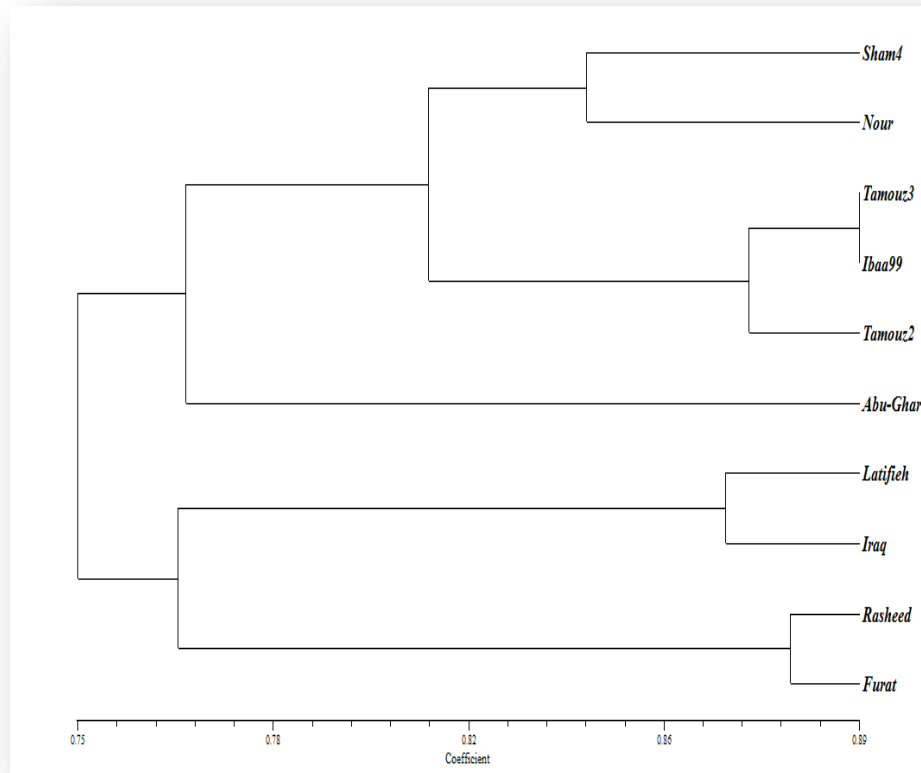


Fig. 1. cluster tree analysis of 10 bread wheat cultivar with 15 SSR primer.

## 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 analyzed of wheat accessions appears genetic diversity more acurity compare with converntional plant breeding methods . the best genetic 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.

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