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Integrated assessment of some productive traits and molecular diversity using ISSR markers in bread wheat

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Abstract

This study aimed to examine the quantitative and molecular genetic variation of four bread wheat (Ibaa99, Furat, Bohooth22, and Mawada), based on yield traits and ISSR markers. The field experiment took place in Najaf Governorate (Iraq), during the 2024 - 2025 winter growing season. It used a randomized complete block design (RCBD) with three replications. Analysis of variance (ANOVA) results showed highly significant differences among the studied varieties in all traits. The Bohooth22 variety recorded the highest values for number of spikes per plant (7.11), thousand-grain weight (43.5 g), number of grains per spike (52.2), and grain yield (32.3 g/plant (. Genetic parameters showed a indicates a relatively stronger genetic control, with phenotypic variation coefficients (PCV) ranging from 6.1% to 23.4% and genetic variation coefficients (GCV) ranging from 4.8% to 19.8%. The traits also exhibited relatively high broad-sense heritability values (68.3% to 71.4%), with relatively high predicted heritability for both grain-to-spike (GA = 7.82; 16.1%) and grain yield (GA = 4.98; 17.3%). The results of the ISSR technique with four primers showed 38 bands. Out of these, 26 were polymorphic, which represents 68% polymorphism. The genetic similarity coefficients ranged from 0.60 to 0.74. Cluster analysis (UPGMA) demonstrated the distinctness of the Bohooth22 genotype and its separation into an independent cluster. These results show the significance of combining quantitative genetics with ISSR markers. This approach helps identify better wheat genotypes that are suitable for breeding programs in Iraq.

Keywords: PCV; GCV; Wheat; PCR; ISSR Markers

1. Introduction

Wheat (Triticum aestivum L.) is the leading cereal crop in Iraq and globally, contributing approximately 20% of calories and 21% of protein to the human diet, making it a pivotal element in achieving global food security. Due to accelerating climate change and increasing demand for its production, boosting and improving wheat yields now largely depends on utilizing the genetic diversity available within cultivated varieties and local genetic resources (FAO, 2021). Genetic variation represents one of the most important biological foundations of approved breeding and genetic improvement programs. The results of many genetic studies have proven that the existence of significant differences in quantitative traits such as the number of grains, the number of fertile spikes, and the thousand-grain weight is often related to true genetic variation and not just to environmental influences. This justifies combining field analysis and molecular evaluation to obtain more accurate results for the genetic makeup of varieties (Mladenov et al., 2021). One of the most important and accurate tools researchers use to assess genetic diversity is molecular markers, due to their independence from the plant's phenological stage or environmental factors. Inter-Simple Sequence Repeats (ISSRs) are among the most important of these markers because they are easy to apply, low-cost, and highly effective at detecting polymorphism in multiple regions of the genome (Zietkiewicz et al., 1994). Numerous studies have demonstrated the efficiency of ISSR markers in analyzing the genetic diversity of wheat. Nagaoka and Ogihara (1997) recorded polymorphism rates of approximately 65–85% when using a limited set of primers. Because of its suitability for ISSR

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data, the Jacquard coefficient is widely used by researchers, followed by clustering analysis using the UPGMA method to produce tree diagrams that illustrate patterns of genetic convergence and divergence, and to analyze relationships between genotypes based on the presence of the band (Etminan et al., 2016).

The combining of the Jaccard coefficient with the UPGMA method provides a reliable representation of genetic relationships in field crops (Roldán-Ruiz et al., 2001). Linking the results of molecular analysis to productive traits is one of the important indicators for researchers, as the study by Alemu et al. (2022) showed a correlation between the molecular variation extracted from ISSR and the significant differences in yield traits and their components in wheat, which supports the use of molecular markers as an aid in selecting genetic parents in breeding and genetic improvement programs aimed at increasing productivity (Alemu et al., 2022). However, a few studies have combined genetic criteria and ISSR-based molecular analysis to characterize the genetic patterns of bread wheat under Iraqi agricultural conditions Based on the above, this study aimed to evaluate the molecular genetic diversity among four wheat varieties (Ibaa99, Furat, Bohooth22, Mawada) using ISSR markers, and to analyze genetic similarity relationships to provide a scientific basis for supporting wheat improvement programs and selecting superior varieties.

2. Materials and Methods

2.1. Genetic material

In this study, four varieties of bread wheat (Triticum aestivum L.) were used, namely Ibaa99, Furat, Bohooth22, and Mawada, coded as V1, V2, V3, and V4, respectively, with the aim of studying the relationship between molecular genetic variation and productive traits.

2.2. Field Design and Experiment Implementation

The field experiment was conducted during the 2024-2025 winter growing season in a farmer's field in Al-Manathira, Najaf. A Randomized Complete Block Design (RCBD) with three replications was used. The varieties were planted on November 10, 2024, in equal experimental units (unit area 2 m^2), and all recommended agricultural practices for wheat (plowing, fertilization, irrigation, and weed control) were applied as described in) FAO, 2021).

2.3. Studied Productive Traits

At physiological maturity, data were recorded on a random number of plants in each experimental unit. The study included the following productive traits (Al-Burki, 2020): number of spikes per plant, spike length (cm), number of grains per spike, thousand-grain weight (g), and grain yield per plant.

2.4. Statistical Analysis

Analysis of variance (ANOVA) was performed for the studied traits using a completely randomized block design to determine if there were significant differences between the varieties. When significant differences were found, the means were compared using the least significant difference (LSD) test at a probability level of 0.05 (Gomez and Gomez, 1984). Statistical analyses were performed using GenStat software (Version 18), Genetic similarity analysis and UPGMA clustering were performed using NTSYS-pc software.

2.5. Estimation of Genetic Parameters

The components of genetic variance ($\sigma^2 g$), environmental variance ($\sigma^2 e$), and phenotypic variance ($\sigma^2 p$) were estimated based on the results of the analysis of variance (ANOVA). The following genetic parameters were calculated according to the rates mentioned previously (Al-Burki, 2020): genetic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in the broad sense (H^2), expected genetic progression (GA) and its percentage. Expected genetic progression was calculated assuming a selection intensity of 5%.

2.6. DNA Extraction

Genomic DNA was extracted from the young leaves of the four varieties using the standard CTAB method. The quality and concentration of the DNA were assessed using agarose gel electrophoresis and spectroscopy (Doyle and Doyle, 1987), and the samples were then stored at −20 °C until use.

2.7. ISSR Analysis

Molecular genetic variation analysis was performed according to the method described by Zietkiewicz et al. (1994) using four ISSR primers (Table 1). Polymerase chain reaction (PCR) reactions were performed at an appropriate reaction volume and included template DNA, an ISSR primer, and a standard reaction mixture.

Polymerase chain reaction (PCR) reactions were performed in a total reaction volume of 25 μL, containing template DNA (≈30–50 ng), one ISSR primer (0.5 μM), a 2× prepared master mix containing Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer, supplemented with deionized water. PCR reactions were performed in a Thermal Cycler according to the following thermal program: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of: denaturation at 94°C for 1 minute, primer fusion at 50–55°C for 1 minute (depending on the primer), elongation at 72°C for 2 minutes, and final elongation at 72°C for 7 minutes. PCR products were separated by agarose gel electrophoresis, and the bands were imaged under UV light. The presence or absence of strips for each category was recorded as a binary matrix (1/0)

Table 1 ISSR primers sequence

Primers name	('Sequence (5'–3
ISSR-1	GATAGAAAGTTACTCAGCT
ISSR-2	AGCTCTGACTTACGCACAAT
ISSR-3	AGCTAGATAGCCAGATTACT
ISSR-4	GCCTGATTGTCTGCGTAACT

2.8. Molecular and Genetic Analysis

The total number of bands, the number of polymorphic bands, and the polymorphism ratio for each ISSR primer were calculated. Genetic similarity coefficients between varieties were also calculated using the Jaccard coefficient. (Rohlf, 2000). Cluster analysis was performed using the UPGMA method, and a dendrogram was plotted to illustrate the genetic relationships between the studied varieties.

3. Results and Discussion

3.1. Analysis of Variance of Yield and Yield Components

Table (2) shows the results of the analysis of variance, which revealed highly significant differences at the probability level $p \leq 0.01$ between wheat varieties in all studied yield traits. The mean squares for the varieties were 1.61 for number of spikes per plant, 1.47 for spike length, 112.5 for number of grains per spike, 37.3 for 1000-grain weight, and 95.8 for grain yield, indicating clear genetic variation among the studied varieties. In contrast, the replicates did not show significant differences, with mean squares ranging from 0.19 to 6.41, and the experimental error values were relatively low (0.32–15.2).

These results indicate that the differences in yield traits are primarily due to genetic differences between varieties and not to environmental influences. These findings are consistent with those of Alemu et al. (2022) who confirmed that the presence of significant differences in the analysis of variance represents an important preliminary indicator of the potential for genetic selection and improvement of wheat productivity.

Table 2 Analysis of Variance (ANOVA) of Some Wheat Yield Traits

Variation source	DF	MS No. spikes	MS Length spikes	MS No. grains	MS weight of 1000 grain	MS Grain Yield
Varieties	3	1.61	1.47	112.5	37.3	95.8
Replicates	2	0.22 ns	0.19 ns	6.41 ns	2.14 ns	4.86 ns
Error	6	0.38	0.32	15.2	5.63	13.8

3.2. Yield and Its Components of Wheat Varieties

Table 3 data show significant differences between the wheat varieties in all traits. Variety V3 significantly outperformed the others, recording the highest number of spikes per plant (7.11) compared to 5.93 in variety V1, the longest spike (11.4 cm) compared to 10.2 cm, and the highest number of grains per spike (52.2) compared to 45.2. This stuff was better, with the highest weight (43.5 g) and yield (32.3 g). It beat variety V1, which only had a yield of 27.2 g, while V2 and V4 were pretty much the same in most traits.

The clear superiority of variety V3 is attributed to its integrated yield components, particularly the increased number of grains and grain weight. Gharib et al. noted this. (2021) It is noted that varieties with higher grain/ear count and thousand grain weight values often achieve higher yields, as the differences between varieties in productive traits primarily reflect their genetic variation and their different responses to environmental conditions.

Table 3 Average productivity traits of wheat varieties

Variety	No. spike\plant	Length Spikes (cm)	No. grains\ spike	Weight of 1000 grain (gm)	Grain Yield
V1	5.93 b	10.2 c	45.2 c	39.3 b	27.2 c
V2	6.39 b	10.6 b	47.5 b	40.7 b	28.4 b
V3	7.11 a	11.4 a	52.2 a	43.5 a	32.3 a
V4	6.38 b	10.8 b	49.5 b	41.1 b	28.0 b
LSD (0.05)	0.60	0.45	2.20	1.80	2.00

3.3. Genetic Variability and Heritability of Productive Traits

The results (Table 4) for the components of variance and genetic parameters indicate a clear superiority of genetic variance (σ^2g) over environmental variance (σ^2e) in all studied traits. The genetic variance values were 0.52 versus 0.21 for the number of spikes per plant, 0.47 versus 0.19 for spike length, 9.61 versus 3.83 for the number of grains per spike, 4.17 versus 1.93 for the weight of 1000 grains, and 6.94 versus 3.01 for grain yield. The broad heritability (H^2) values were pretty high, between 68.3% for the weight of 1000 grains and 71.4% for the number of grains per spike. The genetic (GCV = 4.8–19.8%) and phenotypic (PCV = 6.1–23.4%) variation coefficients were also relatively similar.

The traits we looked at seem to be heavily influenced by genes, because of their heritability values and GCV/PCV ratios. This means we can probably make them better by picking the right ones to breed. Ayana et al. (2023) reported that traits exhibiting moderate to high heritability with limited differences between GCV and PCV are more stable and amenable to genetic improvement in wheat.

Table 4 Components of Variation and Genetic Parameters of Productive Traits

Trait	σ^2g	σ^2e	σ^2p	GCV (%)	PCV (%)	H^2 (%)
No. spike\plant	0.52	0.21	0.73	11.2	13.4	71.2
Length Spikes	0.47	0.19	0.66	6.3	7.6	71.2
No. Grains\ spike	9.61	3.83	13.44	19.8	23.4	71.4
Weight of 1000 grain	4.17	1.93	6.10	4.8	6.1	68.3
Grain Yield	6.94	3.01	9.95	8.7	10.3	69.8

3.4. Expected Genetic Advance for Productive Traits

The expected genetic advance (GA) results in Table 5 show a clear variation among the studied traits when a selection intensity of 5% is applied. The highest expected genetic advance was recorded for the number of spikes per plant, at 1.25 (19.4%), followed by grain yield at 4.98 (17.3%), and then the number of grains per spike at 7.82 (16.1%). The spike length and 1000 grain weight also achieved the lowest average expected genetic progression of 0.90 (8.3%) and then 3.21 (7.8%). The high relative GA values for some traits, particularly number of spikes per plant and grain yield,

indicate the potential for improvement through direct selection, demonstrating the dominance of additive gene action. Sharma et al. (2021) noted that the combination of medium-to-high heritability and high GA is an important indicator of the success of selection programs in wheat. These results are also consistent with the findings of Abd El-Mohsen et al. (2020), who confirmed that yield traits that show high rates of genetic advancement are key targets in wheat breeding programs to increase productivity.

Table 5 Expected Genetic Progression (GA) and its Percentage

Trait	H^2 (%)	GA	GA (%)
No. spike\plant	71.2	1.25	19.4
Length Spikes	71.2	0.90	8.3
No. grains\ spike	71.4	7.82	16.1
Weight of 1000 grain	68.3	3.21	7.8
Grain Yield	69.8	4.98	17.3

3.5. Molecular analysis using ISSR

The results of the molecular analysis in Figure 1 show the amplification products using the ISSR-3 primer. The four genotypes exhibited different band patterns, indicating significant molecular genetic variation among them. These bands included both common and polymorphic bands. This demonstrates the efficiency of the ISSR technique in detecting genetic diversity at the genome level, particularly in regions rich in repeating sequence.

This variation, as shown in Figure 1, is consistent with the quantitative results presented in Table 6. We got a total of 38 bands from the four primers we used. Out of those, 26 were polymorphic, which means our overall polymorphism rate was 68%. The ISSR-3 primer gave us the best polymorphism rate at 72.7%, a bit better than ISSR-1 which was at 70.0%. ISSR-2 and ISSR-4 had lower rates, at 66.7% and 62.5% respectively. The results in Figure 2 indicate that the four primers achieved different variations. The ISSR-3 primer showed significantly better performance in producing these variations compared to the other primers, which is consistent with the figures in Table 6. The results of this study show that there is a medium to high level of genetic variation among the wheat varieties under study, which reflects the reliability of ISSR indices in assessing genetic diversity among different genotypes.

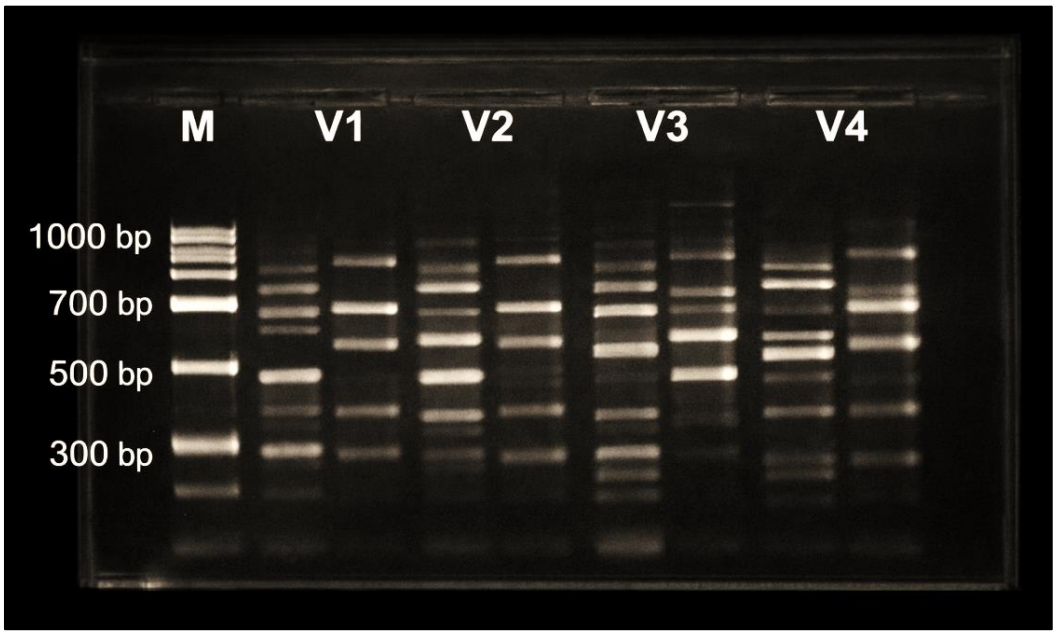

Figure 1 Electrophoretic image on agarose gel of ISSR-3 starter showing polymorphic bundle patterns among wheat varieties. The letter M refers to the DNA ladder

Table 6 Primers used in ISSR analysis and polymorphism ratio

Primer	Sequence (5'–3')	Total number of bands	Polymorphic bands	Polymorphic (%)
ISSR-1	$(GA)_8YC$	10	7	70.0
ISSR-2	$(AC)_8YG$	9	6	66.7
ISSR-3	$(AG)_8YC$	11	8	72.7
ISSR-4	$(GT)_8YG$	8	5	62.5
Total / Average	—	38	26	68.0

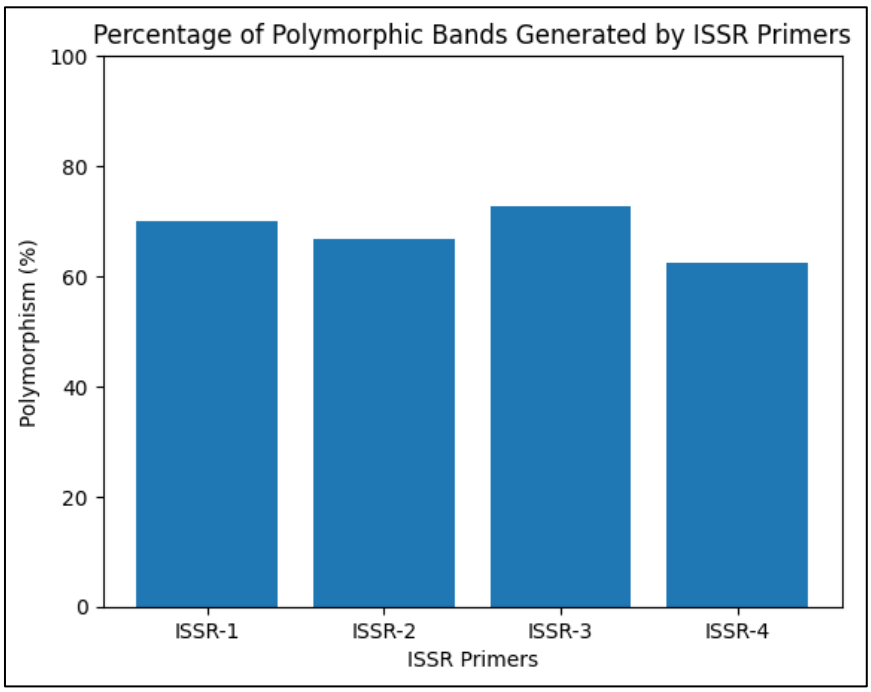

Figure 2 Percentage of polymorphic bands generated by ISSR primers in wheat genotypes

Based on the polymorphism data obtained from ISSR markers, genetic similarity analysis was performed among the studied wheat varieties using the Jaccard coefficient. Cluster analysis was also conducted using the UPGMA method to clarify the genetic relationships between them.

The results of the genetic similarity matrix in Table 7 show that the similarity coefficients between the varieties ranged from 0.60 to 0.74, indicating significant genetic variation. The highest genetic similarity value was recorded between varieties V1 and V2 (0.74), and also between V2 and V4 (0.74), indicating a relative genetic convergence between them. Conversely, the lowest similarity value was recorded between varieties V1 and V3 (0.60), reflecting a clear genetic divergence for variety V3 compared to the other varieties.

The cluster diagram shown in Figure 3 confirms these results, as it shows that variety V3 is clustered in a separate group, while varieties V1, V2, and V4 are grouped together, with a clearer genetic similarity between V1 and V2. This clustering pattern reflects the molecular differences revealed by ISSR markers, indicating that variety V3 has a distinct genetic background compared to the other varieties

These results suggest that ISSR markers are an effective tool for assessing genetic similarity and difference among wheat varieties. They also highlight the potential for utilizing this genetic variation in breeding and genetic selection programs, particularly when selecting genetic parents to improve yield traits.

Table 7 Genetic similarity matrix between wheat varieties

Variety	V1	V2	V3	V4
V1	1.00	0.74	0.60	0.72
V2	0.74	1.00	0.62	0.74
V3	0.60	0.62	1.00	0.62
V4	0.72	0.74	0.62	1.00

Similarity coefficients were calculated using Jaccard's method

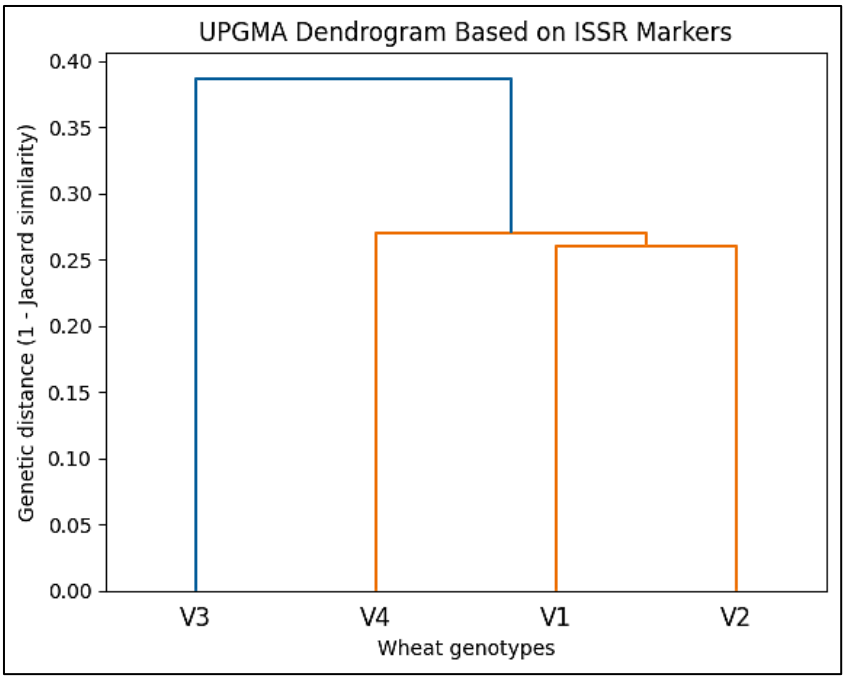

Figure 3 UPGMA dendrogram illustrating the genetic relationships among four wheat genotypes based on ISSR markers using Jaccard's similarity coefficient. The dendrogram reveals the separation of genotype V3 from the remaining genotypes, indicating its distinct genetic background

4. Conclusion

With clear differences in numbers and genes among the wheat types we looked at, there's a lot of genetic variety to work with for making better wheat. Yield traits showed highly significant differences between the varieties, with the Bohooth22 (V3) variety outperforming most yield components and thus achieving the highest grain yield. Genetic parameters demonstrated the superiority of genetic variation over environmental variation, high broad heritability values for most traits, and relatively high expected genetic advancement in spike count/plant and grain yield, indicating the effectiveness of direct selection. ISSR markers confirmed their efficiency in detecting genetic variation among varieties, showing a polymorphism rate of 68%. Using cluster analysis with the Jaccard coefficient, the Bohooth22 variety has a unique genetic makeup. This demonstrates the value of combining numbers with molecular genetics, enabling plant breeders to make more informed choices. Because of this, Bohooth22 could be a good parent to help raise wheat yields.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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