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RESEARCH ARTICLE

Molecular evaluation of several wheat varieties of *Triticum aestivum L.*

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Abstract

This study was carried out with the aim of determining the extent of genetic variation among 10 varieties of wheat. In this work, 5 primers were used to do the PCR to detect the band patterns of ISSR markers. Within the molecular weight range of 100-1500 bp, the 5 primers combined to generate 42 bands, at a rate of 8 bands per primer. Polymorphic bands accounted for 37 of the totals and the ratio of polymorphic bands was 87.97 %. The highest percentage of divergent bands reached 88.88 %, shown by primers HPS13, 842F and HBG20. The starters 1955A recorded a lower rate of efficiency and distinctive ability of the starter reaching 16.22 %. According to the UPGMA method, a phylogenetic tree of the genetic affinity between the varieties was obtained, varieties were separated into 2 major groups: There were 2 varieties in the first major group, namely S7 and S6, 2 minor groups were formed from the division of the second major group. The S3 variant made up the first secondary group, as opposed to the second secondary group. Additionally, it was split into 2 sub-secondary groups: classes S5, S10, S9, S8 and S1 made up the first sub-secondary group and classes S4 and S2 made up the second. Based on the Nei and Li coefficient, the values of genetic similarity between the varieties were estimated, because the S6 variety and the S3 variety had the largest genetic divergence (lowest genetic similarity), with a value of 0.7073 and with the S4 variety, its value was 0.6829, while the highest genetic similarity was (least genetic divergence) between variety S9 and variety S8, where its value reached 0.9268. The findings of this study showed the high efficiency of ISSR makers in detecting genetic variation among several wheat varieties.

Keywords

wheat; ISSR; molecular; DNA; primer; cluster analysis

Introduction

One of the world's most significant cereal crops is wheat *Triticum aestivum* L. because of its strategic role in achieving food security. It provides humans with more than 25 % of calories and protein. It serves as the primary food supply for about 28 % of the global population residing in more than 40 nations (1, 2), as wheat was one of the first plants to be cultivated and scientists believe that farmers planted wheat for the first time about 11000 years ago. The most widely used wheat in Iraq and the world, as the cultivated area in Iraq reached 4.2 million ha, giving a production of 2.6 tons. ha^{-1} (3).

The wheat crop in Iraq suffers from low productivity. This might be because farmers aren't as interested in growing varieties that are characterized by their high yield, especially varieties that adapt to the local environment or due to weak interest in soil and crop service operations. Information related to genetic diversity in varieties is of great importance for improving progeny and obtaining varieties with high yields and resistance to harsh environmental conditions and diseases. Unless there is sufficient genetic diversity between offspring's the probability of obtaining high yields and other desirable traits will be very minimum. Because the selection of improved varieties depends primarily on the genetic differences present in these varieties (4) and to improve the genetic diversity in wheat, it is necessary to know the genetic variations that originally exist in these varieties. Plant breeders have turned to using methods by which it is possible to know the extent of the genetic diversity and closeness between the varieties and for this purpose they have used several techniques, some of which depend on morphological form, some of which depend on proteins and some of which depend on DNA that is extracted from plants for the purpose of obtaining genetic information called DNA markers, as genetic markers through which researchers were able to overcome all the obstacles they faced when using previous methods (5). In recent years and in light of the rapid scientific progress, scientists have been able to discover many modern types of these markers Its work relies on one of the most important modern scientific discoveries, which is the discovery of the PCR reaction, which is the innovation of the Polymerase Chain Reaction (PCR) developed by Kary Mullis, perhaps the most prominent of which is the ISSR markers. One of the metrics that depends on PCR is inter-sequence repeats, which uses primers containing simple repeats of nitrogenous bases to amplify regions between its DNA sequences. It relies on the abundant presence and random distribution of ISSR in the plant genome and is characterized by not requiring prior information about the genome. It results in patterns with large polymorphisms resulting from several genetic sites. To measure genetic diversity, a primer with a sequence based on 3.5 nucleotide sequences can be utilized. ISSR technology is considered an effective technique in detecting genetic variations among plants (4). It is characterized by its high efficiency in detecting Polymorphism, its primers are available and it is one of the fast and accurate techniques in genetic mapping using small amounts of DNA. It is also low in cost and gives high levels of genetic differences between Communities (6). ISSR was used to study genetic diversity among wheat genotypes and showed high efficiency in doing so (7). In a study conducted to distinguish genetic variations between several varieties of wheat, it was found that ISSR primers were effective in detecting these variations (8).

Therefore, this study aims to know the size of the genetic differences between several wheat varieties, that is, to know the degree of similarity and diversity among the variants to identify the elite ones that are distinguished by their high yield and good quality due to their importance in human and animal food.

Materials and Methods

This study used 10 varieties of wheat obtained from the University of Baghdad's Department of Field Crops, College of Engineering Sciences, namely Ibaa 99 (S1), Iraq (S2), Al-Rasheed (S3), Latifiya (S4), Bhooth 22 (S5), Sham 6 (S6), Al-Izz (S7), Babl 113 (S8) Bhooth 10 (S9) and Baghdad 1 (S10). The experiment was carried out on 12/1/2022 in the Al-Bu Shaaban area - north of the city of Ramadi, using the R.C.B.D design with 3 replicates and the analysis of leaf samples collected from 10 cultivated wheat varieties were conducted.

DNA Extraction

Using CTAB (Cety Trimethyl Ammonium), DNA was isolated from plants of cultivars with 5 leaves according to the method (9).

Estimation of DNA concentration and purity

Estimating the purity of the extracted DNA is one of the important and essential steps to ensure that the DNA is pure and of good quality. To quantify and evaluate the DNA, it was electrophoresed on an agarose gel at a concentration of 1 % (10).

DNA Concentration (µ/mL): A 260 x 50

Primers used in this study

This article used 5 ISSR primers obtained from Bioneer - Korea HPS13, 842F, 1955B, 1955A and HBG20 as mentioned in Table (1).

Table 1. Types of primers used in the search and their sequences.

No.	Name	Sequence
1	HPS13	5' GTG TGT GTG TGT CC 3'
2	842F	5' CTCTCTCTCTCTCTCTGC 3'
3	1955B	5' CACACACACACAAC 3'
4	1955A	5' CACACACACACAGT 3'
5	HBG20	5' GAGAGAGAGAGACC3'

ISSR Reactions

ISSR reactions were conducted on DNA samples of the 10 wheat varieties (11). Three processes were included in these reactions: gel separation, amplification and digestion Table (2). The results of the multiplication of ISSR markers were collected by recording images of the patterns of the electrophoresis process for each primer and identifying the bands, in which a DNA band's existence is denoted by the symbol (1) and its absence by the symbol (0). The genetic distance coefficients between the varieties were computed and it was utilized for cluster analysis (12). A phylogenetic tree was drawn and similar individuals were described next to each other in the cluster using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA method) and the genetic distance was calculated based on the following equations:

GD= 1- (2 x (Nij / Ni + Nj)

Table 2. Reaction conditions program of the PCR-ISSR.

Step	Temperature	Time	No. of cycles
Pre- denaturation	95 ˚C	5 min	1
Denaturation	95 ˚C	1 min	
Annealing	50 ˚C	2 min	40
Extension	72 ˚C	1 min	
Final extension	72 ˚C	10 min	1

The percentage efficiency of the primers used was calculated according to the following equation:

Total number of primer bands/ Total number of bands of all primers

The percentage of discriminatory ability for each primer was calculated as follows:

(Number of polymorphic for the primer/ Number of polymorphic for all primers x 100 (13)

Results and Discussion

Table 3 displays the results obtained that the primers HPS13, 842F and HBG20 the greatest number of bands were offered, achieving a total of 9 bands per primer. Of the 42 bands generated by all primers, 37 had different shapes, representing an 87.97 % success rate, as it was recorded Primers HPS13, 842F and HBG20 had the largest number of bands of different shapes, 8, with an 88.88 % success percentage for every primer. The same table's data demonstrated that the primer's maximum rate of efficiency was 21.43 % in HPS13, 842F and HBG20. The highest percentage of discriminative ability was 21.62 % for the same prefixes. These results agreed with what was found (14).

1 - Primer HPS13

9 bands were obtained, including 8 bands of different shapes, which in turn were reflected in the percentage of polymorphic bands, which reached 88.88 %. This primer showed a discrimination ability of 21.62 %. Its efficiency rate was 21.42 %. Table 3 displays the obvious variance in molecular weight and position between 200 and 1500 bp found in the genotypes' genomic DNA that this primer was able to identify through complimentary sequences. It can be seen from Fig. 1 that all genotypes had bands for molecular weights of 100 base pairs and that the rest of the molecular weights' bands appeared in a different way for all genotypes the outcomes agreed with the findings (8, 15).

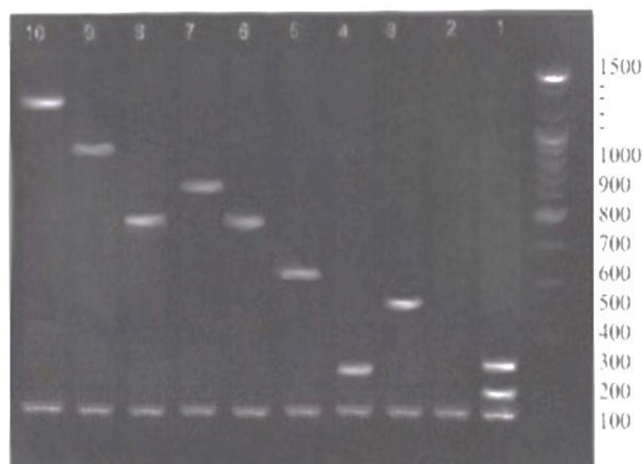

Fig. 1. PCR electrophoresis product of the HPS13 primer on a 1 % M agarose gel.

2 - Primer 842F

8 of the 9 DNA bands that this primer generated were polymorphic, which was reflected as a proportion of polymorphic bands reaching 88.88 %. As for the discriminatory ability of this molecular indicator, it was also about 21.62 % and so was the percentage of efficiency of this primer 21.43 %. As for detecting DNA sequences, Table 3 showed the ability of the primer to reveal the DNA Genome sequencing for genotypes with clear variations in molecular weight from 200 to 1300. From Fig. 2, it is noted that the primer has detected 3 bands. For cultivar Aba 99 (S1) with molecular weights of 100, 200 and 300 bp, these results agreed with another study (16).

Table 3. Shows the primers used, the number of resulting packets, the different packets and their percentages and each primers ratio of efficiency and discriminating ability.

Primers	Resulting bands	Polymorphic bands	Polymorphic bands ratio %	Primer efficiency ratio	Estimated discrimination rate of the primer	Size of DNA bands bp
HPS13	9	8	88.88	21.43	21.62	1500-200
842F	9	8	88.88	21.43	21.62	1300-200
1955B	8	7	87.5	19.05	18.92	1100-200
1955A	7	6	85.71	16.67	16.22	1000-200
HBG20	9	8	88.88 (Average)	21.43	21.62	800-100
Total	42	37	87.97			1500-100

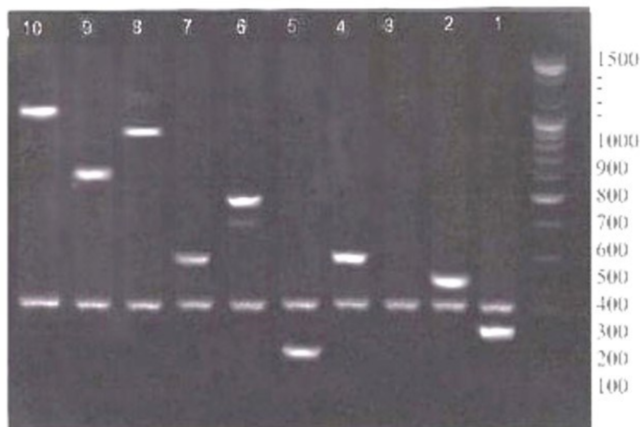

Fig. 2. PCR electrophoresis product of primer 842F on a 1 % M agarose

3 - Primer 1955B

8 bands of DNA were produced, including 7 bands that were polymorphic bands and molecular weight, bringing the percentage of polymorphic bands to 87.5 %. This primer was also efficient in detecting genetic differences, as its efficiency rate reached 19.05 %, while its discriminatory ability was 18.92 %. This primer clearly completes the DNA sequences within the molecular weight of 200 - 1100 (Table 3). From Fig. 3, all of the bands were evidently visible within the molecular weight range of 400 base pairs. The research results were consistent with both (17, 18).

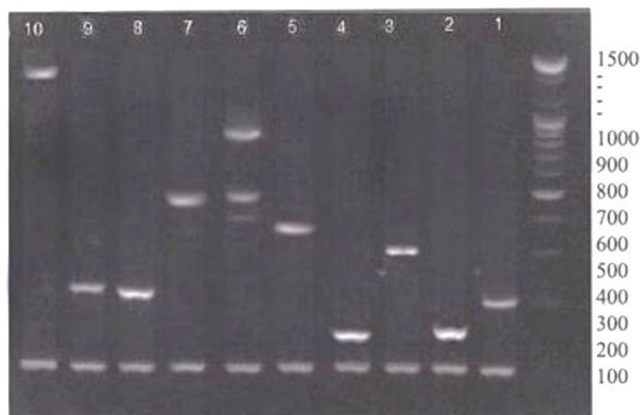

Fig. 3. PCR electrophoresis product of primer 1955B on a 1 % M agarose

4 - Primer 1955A

6 of the 7 bands that this primer generated were polymorphic bands, which led to an increase in the percentage of variance of the bands to 85.71 %, while the percentage of efficiency and discriminatory ability also reached 16.67 % and 16.22 % respectively. Primer sequences appeared weighing between 200 and 1000 base pairs molecularly (Table 3). Fig. 4 shows that divergent bands were observed both at a molecular weight of 200 base pairs and between 400 and 1000 base pairs, they were also divergent. Study results are consistent with (7, 19).

5 - Primer HBG20

The 9 bands produced by the primer were not all identical, but rather 8 of them had polymorphic band, which directly affected the percentage of variation of the DNA bands to

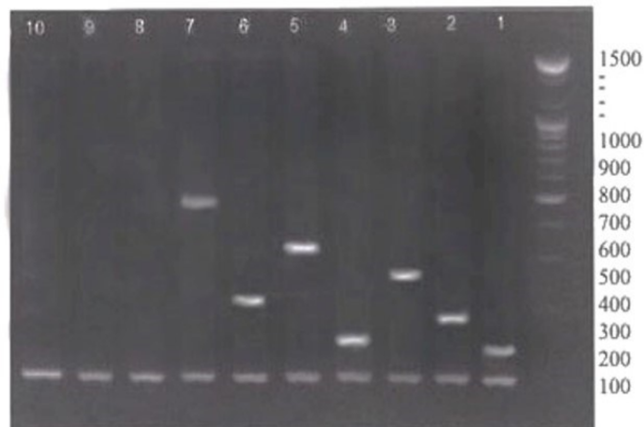

Fig. 4. PCR electrophoresis product of primer 1955A on a 1 % M agarose

reach 88.88 %. The primer showed good efficiency in detecting the bands, as its efficiency rate reached 21.43 %, while its discriminatory ability was 21.62 %. The band variation appeared clearly when the molecular weight is 100-800 base pairs (Table 3). All bands appeared at 100 weights of molecular base pairs (Fig. 5).

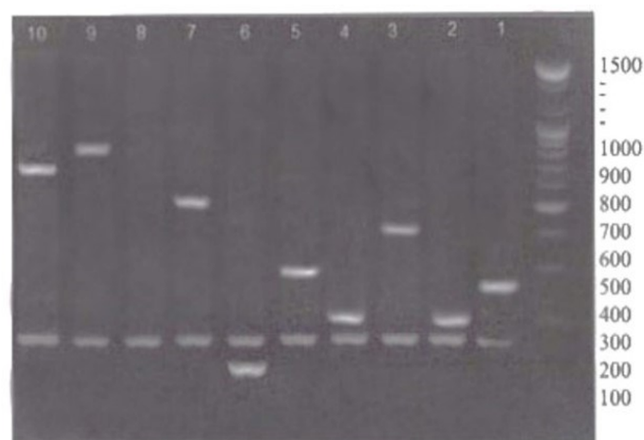

Fig. 5. PCR electrophoresis product of the HBG20 primer on a 1 % M agarose gel.

Genetic diversity values between wheat varieties using ISSR primers

When determining the genetic diversity rates among the wheat varieties, the results in Table 4 showed the presence of 37 polymorphic bands, using the Nei and Li coefficient, as can be seen from Table 4, there was a greater genetic divergence among the S6 and S3 varieties and its value was 0.7073 and with the S4 variety, its value was 0.6829, while the highest genetic similarity was (least genetic divergence) between variety S9 and variety S8, where its value reached 0.9268. Therefore, genetically divergent and superior varieties can be introduced into a successful breeding program, especially the hybridization program and then followed by selection to reach a variety that includes all the good indicators in one variety to be promising. The results were identical to (20, 21).

Draw a genetic Phylogenetic tree of wheat varieties based on ISSR markers

All that the genetic tree represents is an evolutionary relationship between several species and the branches are represented in the form of branches of this tree and the branches close to each other represent the degree of

Table 4. Genetic similarity values between the studied wheat varieties.

	S.1	S.2	S.3	S.4	S.5	S.6	S.7	S.8	S.9	S.10
S.1										
S.2	0.7561									
S.3	0.7317	0.7805								
S.4	0.8049	0.9024	0.7317							
S.5	0.7561	0.8049	0.7805	0.7561						
S.6	0.6829	0.7317	0.7073	0.6829	0.7317					
S.7	0.7317	0.7805	0.7561	0.7317	0.7805	0.8049				
S.8	0.878	0.878	0.8537	0.8293	0.878	0.8049	0.8537			
S.9	0.8049	0.8049	0.7805	0.7561	0.8049	0.7805	0.7805	0.9268		
S.10	0.7561	0.8049	0.7805	0.7561	0.8049	0.7317	0.7805	0.878	0.8049	

similarity between the species (22). The results of the cluster analysis of ten wheat varieties using ISSR primers according to the UPGMA approach in Fig. 6 revealed that the 10 varieties could be divided into 2 primary groups: the first main group consisted of 2 varieties, namely S7 and S6. Two subgroups emerged from the division of the second major group. The S3 variety made up the first secondary group. Two sub-secondary groups were also created from the second secondary group. The first sub-secondary group consisted of 2 varieties, namely S4 and S2 and the second sub-secondary group consisted of varieties S5, S10, S9, S8 and S1. These results agreed with another findings (23).

Conclusion

The ISSR markers were highly efficient in detecting genetic variations between varieties, with the highest primer efficiency ratio reaching 21.43 in 3 primers out of the 5 primers used in this study. ISSR indicators can be used to determine genetic variations between varieties before performing genetic crosses in order to produce synthetic varieties with high yields and other desirable traits.

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Authors' contributions

ASH and FHM brainstormed and designed experiments as well as performed the experiments. ALMA, MOM, SSS AT and ASH wrote the article and designed it.

Compliance with ethical standards

Conflict of interest: None

Ethical issues: None

References

1. FAO. Save and grow in practice: maize, rice, wheat. 2015; ISSN: 978- 92- 5- 108519-6p124.
2. Abdul Kafoor AH, Ahmed YA, Ali IM, Ramadan ASAA. Growth and yield response of several cultivars of wheat *Triticum aestivum* L. to spraying of NPK nano fertilizer. In: IOP conference series: Earth and Environmental Science. 2023;1252(1):p. 012033. https://doi.org/10.1088/1755-1315/1252/1/012033 IOP Publishing. https://iopscience.iop.org/article/10.1088/1755-1315/1252/1/012033

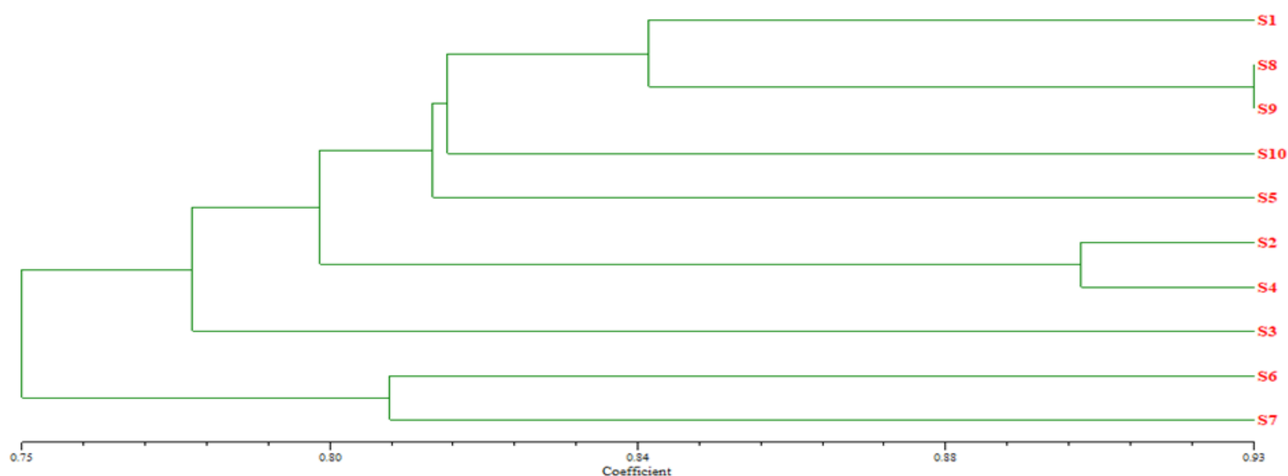

Fig. 6. Diagram of cluster analysis based on ISSR marker.

3. Ministry of Planning. Central Bureau of Statistics - Agricultural Statistics; 2015. https://mop.gov.iq/en/central-statistical-organization
4. Al- Obaidi SSM. Morphological and molecular evaluation of genetic variation for number maize *Zea mays* L. inbred lines [Unpublished doctoral dissertation]. Field Crops Department. College of Agriculture. University of Anbar. 2018. https://doi.org/10.32649/aagrs.2018.153334
5. Mukhlif FH, Ramadan ASA, Hammody DT, Mousa MO, Shahatha SS. Molecular assessment of genetic divergence among maize genotypes. Sabrao Journal of Breeding and Genetics. 2023;55(3) 739-48. https://doi.org/10.54910/sabrao2023.55.3.12 https://sabraojournal.org/molecular-assessment-genetic-divergence-among-maize-genotypes/
6. Vijayan K, Anuradha HJ, Nair CV, Pradeep AR, Awasthia AK, Saratchandr BS, et al. Genetic diversity and differentiation among populations of the Indian silkworm, *Samia cynthia* ricini, reversed by ISSR markers. J Insect Sci. 2006;6:11-30. https://doi.org/10.1673/2006_6_30.1. https://pubmed.ncbi.nlm.nih.gov/19537974/
7. Abouseada HH, Mohamed AH, Teleb SS, Badr A, Tantawy ME, Ibrahim SD, et al. Genetic diversity analysis in wheat cultivars using SCoT and ISSR markers, chloroplast DNA barcoding and grain SEM. BMC Plant Biology. 2023;23:193 https://bmcplantbiol.biomedcentral.com/articles/10.1186/s12870-023-04196-w
8. Abdulhamed ZA, Hwaidi MI, Alqaisi MRM. Determination of maize genotypes performance under water deficit using ISSR molecular index. Plant Science Today. 2023;10(1):30-37. https://www.researchgate.net/publication/365071907_Determination_of_maize_genotypes_performance_under_water_deficit_using_ISSR_molecular_index
9. Weigand F, Baum M, Udupa S. DNA molecular marker techniques, technical manual. No. 20. International Center for Agricultural Research in Dry Area (ICARDA). Aleppo- Syria; 1993. https://mel.cgiar.org/reporting/download/hash/05298131b447d28258a2fd3c34ecefc0
10. Weising K, Atkinson RG, Gardner RC. Genomic fingerprint by microsatellite - Primed PCR: acritical evaluation. PCR Methods and Application. 1995;4:249-55. https://doi.org/10.1101/gr.4.5.249. https://genome.cshlp.org/content/4/5/249
11. Williams JG, Kubelic AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990;18:6531-35. https://doi.org/10.1093/nar/18.22.6531 https://genome.cshlp.org/content/4/5/249
12. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceeding of the National Academy of Sci. 1979;79:5269-73. https://doi.org/10.1073/pnas.76.10.5269
13. Machugh DE, Loftus RT, Bradley DG, Sharp PM, Cunningham P. Microsatellite DNA variation within and among European cattle breeds. Proc Biol Sci. 1994;256:25-31. https://doi.org/10.1098/rspb.1994.0044. https://royalsocietypublishing.org/doi/10.1098/rspb.1994.0044
14. Khodadadi E, Farokhzadeh S. Assessing genetic diversity of indigenous bread and durum wheat cultivars in Iran using inter simple sequence repeat (ISSR) markers. Genetic Resour Crop Evol. 2024;71(6):1-16. https://doi.org/10.1007/s10722-023-01835-x. https://link.springer.com/article/10.1007/s10722-023-01835-x
15. Al-Naggar AMM, Soliman AM, Hussien MH, Ibrahim SD, Mohamed EMH. Genetic relatedness among maize inbred lines based on ISSR markers and its association with heterosis and hybrid performance. Innovare Journal of Agricultural Science. 2022;10(6):5-9. https://doi.org/10.22159/ijags.2022.v10i6.46548 https://journals.innovareacademics.in/index.php/ijags/article/view/46548
16. Abdul Rehman RG, Atta Ullah AN, Noor H, Jan A, Faisal S, Reki AR. Phenotypic and molecular confirmation of Maize (*Zea mays* L.) genotypes for drought tolerance at seedling stage. Pure Appl Bio. 2023;12(4):1548-55. https://doi.org/10.19045/bspab.2023.120156 https://www.thepab.org/files/2023/September-2023/PAB-MS-2307-079.pdf
17. Amoon MH, Abdul - Hamed ZA. Determination genetic diversity of inbred lines and hybrid of maize using ISSR technic. Iraqi Journal of Agricultural Sciences. 2020;51(1):269-77. https://doi.org/10.36103/ijas.v51i1.925 https://jcoagri.uobaghdad.edu.iq/index.php/intro/article/view/925
18. Mohamed Ahmed EM, El Hussein AA, El Siddiq MA. ISSR profiles and protein patterns related to yield and yield compositions in several bread wheat genotypes growing under thermal stress. Journal of King Saud University- Science. 2024;36(4). https://doi.org/10.1016/j.jksus.2024.103139 https://sabraojournal.org/qualitative-characteristics-genomic-analysis-wheat-genotypes-iraq/
19. Nosair HR. Genetic diversity studies on seven Egyptian wheat (*Triticum aestivum* L.) cultivars using Scot and ISSR polymorphism markers. Taeckholmia. 2020;40:143-51. https://doi.org/10.21608/taec.2020.39905.1025 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC413122/
20. Shaban AS, Arab SA, Basuoni MM, Abozahra MS, Abdelkawy AM, Mohamed MM. SCOT, ISSR and SDS- PAGE investigation of genetic diversity in several Egyptian wheat genotypes under normal and drought conditions. International Journal of Agronomy. 2022. https://doi.org/10.1155/2022/7024028 https://www.hindawi.com/journals/ija/2022/7024028/
21. Ismail EN, Majeed DM, Al Agely, Abed AS, Hussien GA. Qualitative characteristics and genomic analysis of wheat genotypes in Iraq. Sabrao Journal of Breeding and Genetics. 2023;55(3):896-906. https://doi.org/10.54910/sabrao2023.55.3.24. https://sabraojournal.org/qualitative-characteristics-genomic-analysis-wheat-genotypes-iraq/
22. Al-Faraji Hamadi, Mukhlif Hamdi Jassim Fadhil Hussein, Hassan Ibrahim Ismail. Estimation of gene action, genetic diversity and hybrid vigour in lines of maize (*Zea mays* L.) using the SSR technology [Doctoral dissertation]. University of Anbar-Collage of Agricultural Department of Crops Science. .2020 http://194.127.137.90:8080/repoAnbar/handle/123456789/9012
23. Barman M, Choudhary VK, Singh SK, Parveen R, Roy A, Debbarma P. Cluster analysis in bread wheat (*Tricitum aestivum* L.) genotypes for morphophysiological traits and grain micronutrient content. The Pharma Innovation Journal. 2021;10(5):1588-91. https://www.thepharmajournal.com/archives/?year=2021&vol=10&issue=5&ArticleId=6458