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Genetic Diversity In Maize Genotypes Using ISSR Markers

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Abstract
This work studies how much genetic diversity exists among five maize genotypes using inter-simple sequence repeat (ISSR) markers. Altogether, there were 110 bands, with an average of 22 alleles found for each primer used. The genetic similarity coefficient analysis came out to 0.69 and values ranged from 0.39 to 0.68, that means there is variation in genetic similarity between maize genotypes. Results reveal that ISSR markers help identify genetic diversity in maize varieties and this data can assist in the planning of programs for improving maize seeds.

Keywords
Genetic Diversity, Maize, ISSR Marker, Genetic Similarity, Polymorphism, Genotype

1. INTRODUCTION

Maize which is called Zea maysL. by scientists, is very important for the world's food and livestock supplies. In many developing countries, maize provides a main source of food to a large part of their population (Flint-Garcia, 2013). Because maize needs to be more productive and resilient to risks from the environment, it is necessary to thoroughly study the genetic diversity existing in maize (na et al., 2020). Many experts point out that the current breeding techniques have capability gaps when it comes to mixing genetic and environmental aspects of morphology (Kumar et al., 2023). Some small advances in molecular biology such as using Inter-Simple Sequence Repeat markers, are now being utilized to handle genetic problems in plants (Kumar et al., 2023). The objective is to investigate how five types of maize differ genetically by using ISSR markers (Contreras-Soto et al., 2021). Central America was where maize, an annual C4 plant from the Poaceae family, first grew and it is now a popular cash crop contributing greatly to the world's coarse grain trade (Andorf et al., 2019). It is a major source of nutrition for about 900 million people without much money, 120-140 million poor farmers and almost one-third of all malnourished children across the world (according to Shikha et al., 2021).

Many refer to maize as the "Queen of Cereals" or the "Miracle Crop" for its important position in global agriculture. It is majorly grown around the world because its genetic yield is higher than that of other cereals. Sugarcane produces more carbohydrates per unit area per day than any other crop which is why it's a productive food crop (Balasundara et al., 2021). Besides being eaten by people and animals, maize gives us important ingredients for different farming businesses, like baking, producing seeds for human food, oil and animal feed. Furthermore, corn starch is used in the production of paper, cardboard, textiles and different types of products. More researchers are using ISSR markers in plant studies because they are easy to find, have good reproducibility and contain useful information (Kumar et al., 2023). This way of studying enables scientists to directly examine genetic material at the DNA level, providing a clear picture of genotypes' genetic differences. Although maize is known to have a lot of genetic variation, the diversity of maize landraces is rarely looked into (Wang et al., 2018). Variety among crops is necessary for them to survive stresses and meet the increasing needs of a bigger population around the globe.

LITERATURE REVIEW

Using markers helps in crop breeding and is based on genomic DNA. Thus, maize breeders need methods that quickly and affordably process many samples. Many existing techniques for DNA extraction are not very efficient and they use costly chemicals, stopping many genetic studies and breeding projects (Bakari, 2015). For these reasons, miniprep DNA extraction procedures suitable for limited labs, including those without liquid nitrogen, have become more popular and help produce high-quality DNA isolated from maize leaves (Kumar et al., 2023). A majority of the times, these approaches result in DNA that has the proper purity and can be used in PCR and further analyses (Magadmi et al., 2023). Improving these

procedures increases the use of molecular markers in maize breeding which results in faster identification and pick out of desirable traits (Andorf et al., 2019).

n farmers, the way genetic diversity is assessed is mostly by using DNA markers and the system used is different, depending on the purpose of the study, what can be afforded, who is capable and the resources available (Contreras-Soto et al., 2021). New methods in DNA analysis have greatly increased what we can understand about evolution and have offered strong answers to tough questions on reproduction, speciation and changes in population numbers (Contreras-Soto et al., 2021). Microsatellites which are also called simple sequence repeats, are used often in genetic diversity studies of maize due to their many positive qualities. The process with microsatellites is reliable, uses little DNA, shows a wide range of differences and gives important genetic information, even with similar varieties. Because they are present in large numbers, easy to examine and strongly dominant, they can play many roles in maize research. Thanks to microsatellites, it has become possible to make detailed genetic maps in maize and to map as well as select for important agronomic traits using markers (Sharopova et al., 2002).

1.1 Objective

In this study, we want to assess genetic diversity among five maize genotypes with the help of ISSR markers. The particular goals include:

- To isolate DNA from the sampling of maize lines.
- To determine the genetic similarities among five types of maize with the help of ISSR markers.
- To predict how much variation and commonality there is in the maize genotypes.

1.2 Significance

Gaining insight into maize's genetic variation is important for selecting the best genotypes for breeding. Judging genetic variation fast using molecular markers can improve the success rate of breeding. This study is intended to find valuable genetic differences in maize and assist with improving maize.

2. MATERIALS AND METHODS

2.1 Experimental Details

This study was conducted to assess the genetic diversity among five maize (Zea mays L.) genotypes using ISSR markers. The maize varieties selected for the study were:

1. **Rashi-3494**
2. **Rashi-4750**
3. **Kaveri-3712**
4. **Asian-1232**
5. **Syngenta-6524**

These genotypes were chosen based on their agronomic importance and availability for genetic diversity analysis.

2.2 DNA Extraction

Maple leaves were used to obtain genomic DNA and the CTAB method was applied. To start, a preparation of leaves in liquid nitrogen was mixed with the DNA extraction buffer. After the eggs had been incubated, the aqueous phase was separated from the butyl acetate by adding chloroform: isoamyl alcohol (24:1). Isopropanol was used to precipitate DNA and afterward it was washed with ethanol before being resuspended in TE buffer. Checking the quality of the DNA was done by agarose gel electrophoresis.

2.3 PCR Amplification Using ISSR Markers

Five ISSR primers were used to amplify the genomic DNA from the maize genotypes (Table 3.6). The PCR reactions were performed with the following reaction mixture:

- 1 µl of template DNA (50 ng/µl)
- 5.75 µl of ddH2O
- 1 µl of 10X PCR buffer
- 1 µl of dNTPs (1 mM each)
- 1 µl of primer (10 µM)
- 0.25 µl of Taq polymerase (1 U/µl)

The amplification was carried out using a thermal cycler with the following temperature profile:

- Initial denaturation: 95°C for 5 minutes
- Denaturation: 95°C for 30 seconds
- Annealing: 54°C for 1 minute

- Extension: 72°C for 1 minute
- Final extension: 72°C for 7 minutes
- Hold: 4°C for 24 hours

PCR products were visualized by 2% agarose gel electrophoresis, stained with ethidium bromide, and documented using a Gel Doc system.

2.4 Data Analysis

The PCR results were investigated for differences and variation. Each band was given a score: 1 for presence and 0 for absence and these scores were used to create a binary matrix. Using the Jaccard coefficient, genetic similarity was found and visualized in a dendrogram that was produced by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis.

2.5 Estimation of Polymorphism Information Content (PIC)

To see how informative the ISSR markers were, the polymorphic information content (PIC) was determined with this equation:

$PIC_i = 2f_i(1-f_i)$

f_i= the band is present and $1-f_i$ indicates the band is absent.

2.6 Cluster Analysis

The color varieties of maize were sorted into groups based on their genetic similarity with UPGMA clustering done via the NTSYS-pc software. It revealed how the genotypes were different and how they were connected with each other.

3. RESULTS

It Is Necessary To Extract DNA From Available Plant Materials such As The Leaves Of Maize Cultivars.

DNA extracted from plant tissues will have differing methods for various materials. Any technique that can break the cell wall and membranes, letting you reach the nuclear material, without harming it is needed for this process. Sufficient genomic DNA was gathered to run several PCR-based assays in the investigation. Isopropanol and dry ice bring out DNA precipitation or stiff DNA that appears like thin threads and the pellet obtained at the end of centrifugation is translucent or white (Figs. 3.1 and 3.2)

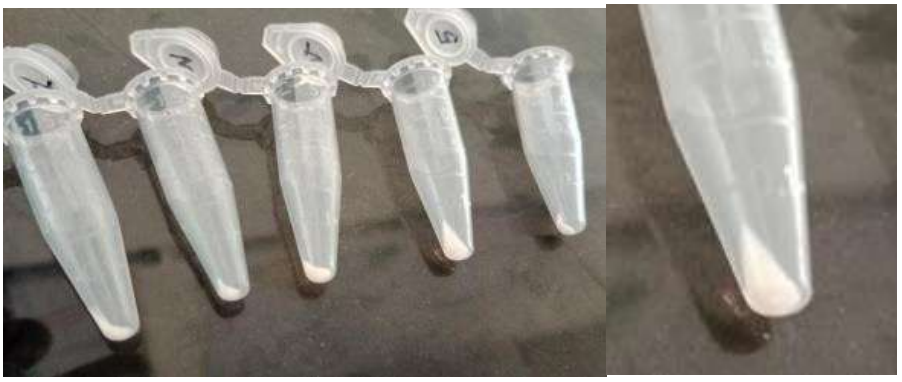
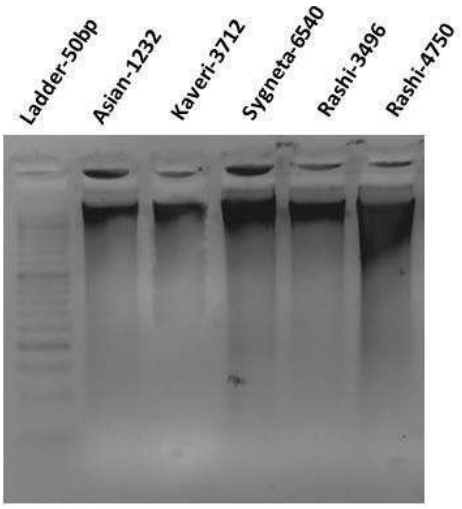


Fig. 3.1: DNA precipitation

Fig. 3.2: DNA pellet of transparent to white colour

In the investigation happening now, the extracted DNA is checked on an agarose gel and then the gel is stained with ethidium bromide and the DNA samples are viewed under UV light. After doing agarose gel electrophoresis on genomic DNA, bright and discernible fragments were revealed.

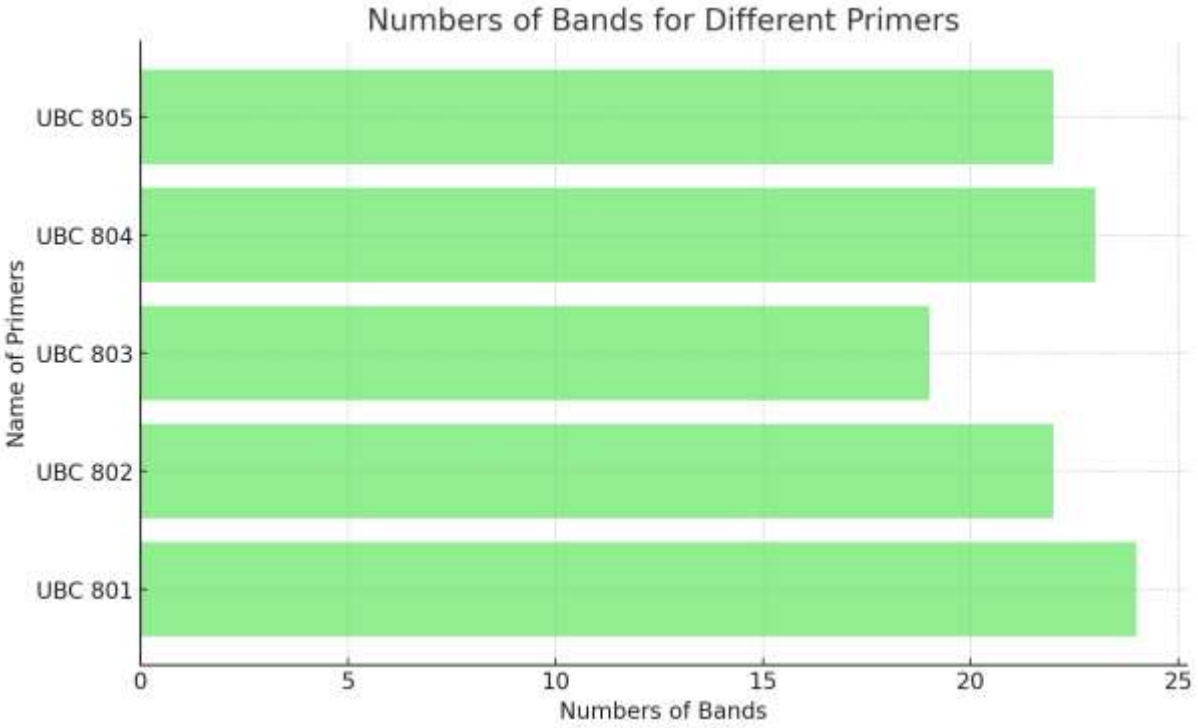
Fig. 3.3: Agarose gel electrophoresis of genomic DNA



No RNA contamination was seen in all the samples, but the presence of degraded DNA was detected in the sample Rasi-4750 (Fig. 4.3). The DNA standard or ladder divided enough to make it possible to tell the size of the different bands in the sample.

Table 3.1: Details of ISSR markers with their range of product size, total number of amplified bands and their polymorphic (P) or monomorphic (M) nature in Maize genotypes

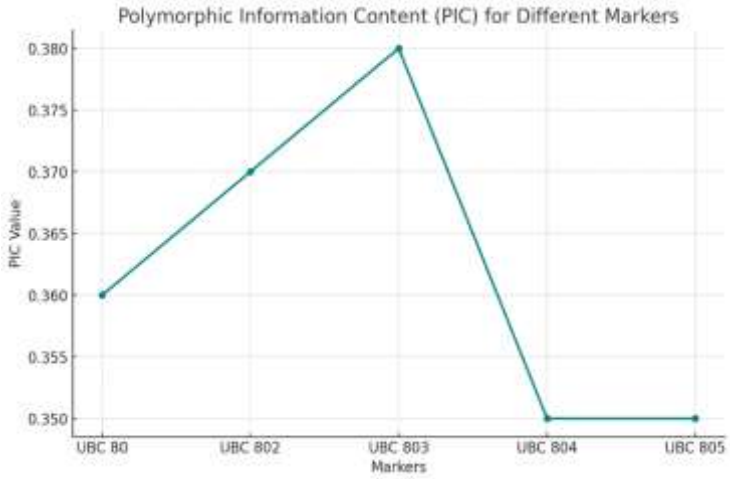
S. No	Name of primers	Range of product size (bp)	Numbers of bands	M/P
1	UBC 801	180-550	24	P
2	UBC 802	180-550	22	P
3	UBC 803	180-550	19	P
4	UBC 804	250-800	23	P
5	UBC 805	180-550	22	P
M= Monomorhic and P= Polymorphic				



In order to get the results, 5 ISSR markers were used and the product size (bp) and total number of bands are shown in Table 3.1. The length of many amplified bands was between 100 bp and 800 bp. The diversity of Maize in 5 different genotypes was studied using the 5 ISSR primers. The number of bands for each primer ranged from 19 (UBC 803) to 24 (UBC 802) and the average was 22 bands created by one primer.

Table 3.2: Polymorphic information content (PIC) based on scoring of polymorphic ISSR markers used in marigold genotypes

S. No.	*Markers*	Size of bands	PIC
1	UBC 80	180-550	0.36
2	UBC 802	180-550	0.37
3	UBC 803	180-500	0.38
4	UBC 804	250-800	0.35
5	UBC 805	180-550	0.35



The gradient line graph displaying the Polymorphic Information Content (PIC) for each marker. The line connects the PIC values, with markers at each data point, showing the trend for the different markers.
Using the PIC index to see how many polymorphic bands a primer creates has often been applied in several genetic diversity studies. How many detectable alleles there are and how they are distributed determines the results which is the same as measuring gene diversity. The UBC 803 marker among ISSR revealed the greatest PIC value, that is, 0.38, implying that ISSR is highly reliable and useful for identifying different Maize genotypes on the molecular level (Table 3.2).

3.4 GENETIC VARIABILITY OF MAIZE GENOTYPES BASED ON ISSR MARKERS

Right now, the dendrogram is made using the genetic similarity matrix which comes from the UPGMA cluster analysis. The 'X' and 'Y' clusters are found, with Rashi- Ashian , Syegenta and Kaveri belonging to group 'B' marked as cluster X. Because they both had the highest genetic similarity (0.68), the Maize genotypes Asian and Sygenta were grouped within the one cluster 'X'. Group 'A' (Y) includes only the genotype Rashi-3496 which stands for the highest difference from the other genotypes included in this study. All in all, the members of the genotypes in my study tended to be genetically similar.

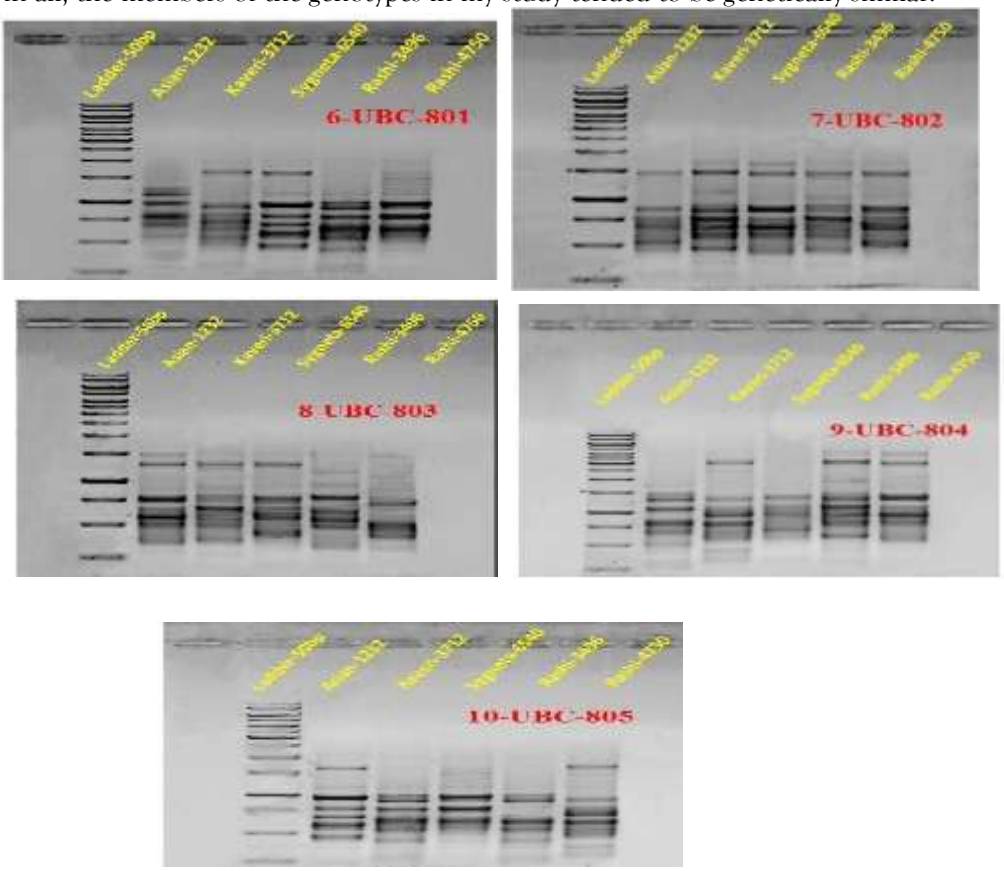


Fig. 3.4: PCR amplification products obtained with ISSR primers. Lane first represents 100bp marker and remaining lanes represent Maize genotypes

To find out the diversity in the present investigation, DNA of 5 genotypes of Maize were subjected to PCR with primers (ISSR). The bands that give clear results on the ISSR markers were used for development of the scoring data. During scoring, the presence of a band gets a mark of '1' and the absence of bands in genotypes is marked '0'. The results of my study proved that the ISSR primers (5) produced effective and dependable patterns of bands. In addition, each genotype's DNA was used in the PCR amplification process. Subsequent to conducting gel electrophoresis, the gel image was used to assign '0' and '1' by comparing with the 100 bp DNA ladder (Fig. 3.4).

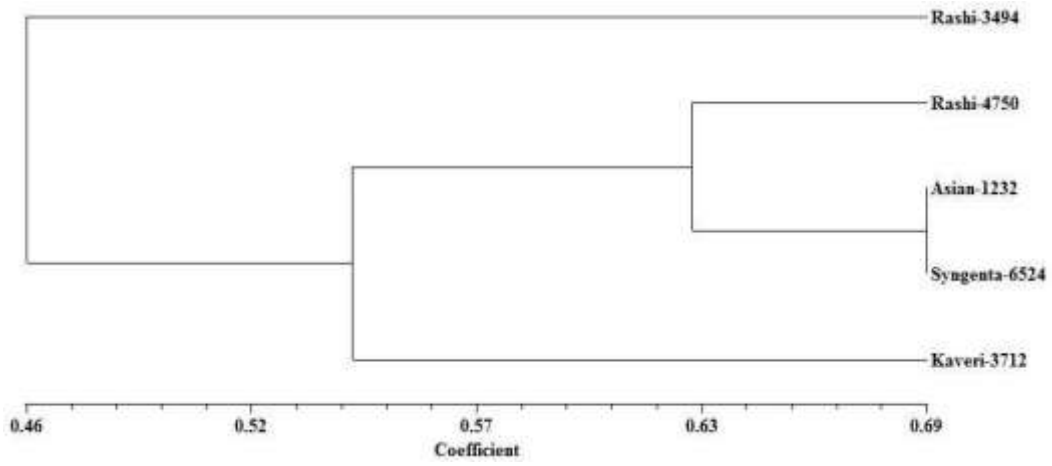


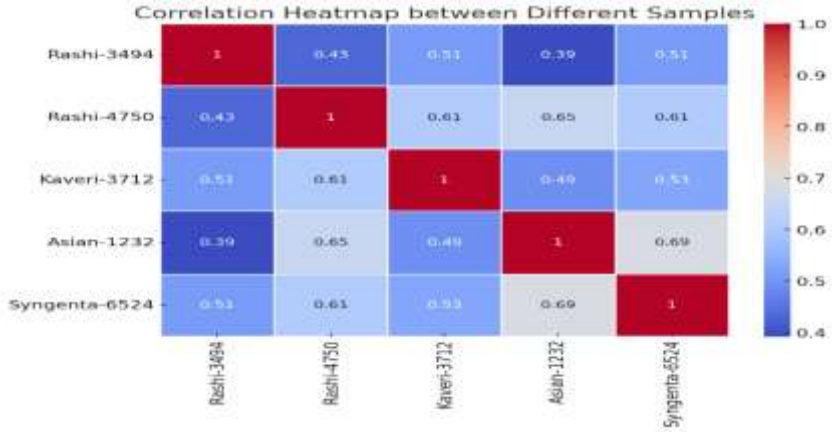
Fig. 3.5: UPGMA based clustering of Maize genotypes using genetic similarity matrix developed from ISSR markers

3.1 EVALUATION THE GENETIC RELATIONSHIPS AMONG FIVE MAIZE CULTIVARS BASED ON ISSR MARKERS

In the present investigation, we will find the details on genetic complexity or variability and how such properties aid in choosing diverse genotypes. Besides the different PCR markers, one of the common markers used for genetic diversity analysis is ISSR and this system relies on variations seen in DNA sequences by using different primers. In this study, the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) was used to cluster the dendrogram and it is popular for displaying the patterns in the groups of individuals.

Table 3.3: Genetic similarity matrix based on ISSR markers data

	Rashi-3494	Rashi-4750	Kaveri-3712	Asian-1232	Syngenta- 6524
Rashi-3494	1.0000				
Rashi-4750	0.4314	1.0000			
Kaveri-3712	0.5098	0.6078	1.0000		
Asian-1232	0.3922	0.6471	0.4902	1.0000	
Syngenta-6524	0.5098	0.6078	0.5294	0.6863	1.0000



The heatmap representing the correlation matrix between the different samples. The colors indicate the strength of correlation between the samples, with annotations showing the exact correlation values. The data for molecular markers was looked at to produce a similarity matrix for each type of genotype. When looking at various Maize genotypes, the mean calculated value was 0.69 and it lay in the range of 0.39 to 0.68. Sygenta 6524 and Asian 1232 showed the maximum similarity among all the genotypes (0.68); therefore, we conclude that they are very similar at the genetic level. In addition, pairs made up of Asian 1232 and Rashi 3496 were found to be very varied, as these genotypes had the lowest genetic similarity (0.39), according to Table 3.3.

4. DISCUSSION

Analysis of this study proves that the five maize genotypes are highly diverse at the genetic level, something that previous research using molecular markers has also shown (Carvalho et al., 2002; Schlotterer, 2004). It appears that the use of ISSR markers is very helpful for seeing differences and similarities among related maize varieties. Since genetic diversity was found in this study, it means maize breeders can create new varieties with better traits such as being resistant to diseases, withstanding drought and yielding more. Because ISSR markers display a lot of variation, they can be used successfully for both gene mapping and the preservation of plant germplasm.

6. Limitations

There were some limitations in this study despite ISSR markers demonstrating good potential for assessing the genetic diversity of maize genotypes. Because only five genotypes were tested, the results may not be useful for all maize varieties. If the sample size were larger, it would be possible to conclude more sure things and explore more forms of maize diversity. Also, ISSR markers have populations with a wide variety of features and can be applied to different situations; however, they are still mainly used as qualitative makers. Hence, in the future, genetic studies could add co-dominant features such as microsatellites (SSRs), to collect more accurate and quantitative information regarding genetics. Finally, temperatures, soil conditions and water being available in the area could have affected the genetic diversity, but they were not analyzed in this study. More investigations might focus on whether environment plays a role in shaping genetic changes.

7. Future Directions

Further research can be done in many ways based on the findings of this study. An initial step to achieve this would be to test more maize strains from various locations which would accurately represent the genetics in the maize population. Furthermore, pairing ISSR information with SSRs and SNPs may give a better picture of the genetic makeup of maize. It would also be helpful to apply these molecular markers for trait mapping so that we can find the genes involved in traits like drought tolerance, resistance to diseases and higher yields. Also, further analysis could be done on how selection based on genetic diversity helps maize develop resistance to the tough conditions caused by climate change.

8. Ethical Considerations

All the activities in this research are guided by ethical standards for science. The study included only plant material taken from government agricultural research farms and public germplasm banks. Everything in

the research happened with the proper permission from all the concerned authorities. The study was carried out according to the standards for molecular biology research; therefore, each process like DNA extraction, PCR amplification and examination of the data was conducted with honesty. Research projects involving people, animals or sensitive areas in the future will always obey the ethical review process.

9. CONCLUSION

Genetic variations among five maize samples were determined by ISSR markers in this study. The findings suggest that there is a lot of genetic diversity among the types of maize which could be beneficial for breeders in the future. Using ISSR markers is a quick, budget-friendly and reliable approach to assess genetic diversity and boost the results of breeding programs.

It would be valuable to apply ISSR markers in wider groups of maize populations to learn more about their genetics and identify what traits should be improved.

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