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Molecular Characterization of Maize (*Zea mays* L.) Inbred Lines using Simple Sequence Repeats (SSR) Marker

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Genomic DNA was isolated by CTAB method and then subjected to PCR amplification by using 22 SSR primers. Among 22 primers, 17 primers were amplified. The PIC values were ranged from 0.46 (Phi046) which is the minimum value to 0.81(Phi059) which is the highest value. The frequencies of alleles were ranged from 0.01 to 0.07. By the use of Unweighted Paired Group Method (UGPMA) for cluster analysis and formed two cluster. DHM117 showed 65per cent similarity with CLQRCY44 and CLO2450 showed 3per cent similarity with BML-6 which is the minimum value. In this study, PIC value indicated a good efficiency of marker for studying the polymorphism level available in studied inbred line and it also indicated their suitability for further breeding programs.

Introduction

Maize (*Zea mays* L., 2n=20) in America and India is referred to as corn which literally mean "that which sustains life" (Akinyele and Adigum, 2006). It belongs to gramineae family. It is the third most important crop in India after rice and wheat. It is grown worldwide in a wider range of environments because of its greater adaptability (Kogbe and Adediran, 2003). Globally, maize is known as Queen of cereals because it has highest genetic yield potential among the cereals. Grain provides food items which are consumed in the form of starch which accounts 14per cent, corn flakes also glucose.

It is also used as animal feed in poultry which accounts 47per cent and it is the most important use and demand of maize. Farmers can easily shield the deteriorating grade of soil by growing the maize which preserves 90per cent of water and 70per cent of potency and it is more profit than wheat and paddy in

production. Simple Sequence Repeat (SSR) marker also known as microsatellites. It is a random repeated motifs of 1-6 nucleotides present in all eukaryotic and prokaryotic genomes and coding and non-coding nuclear and organeller DNA (Zane *et al.,* 2002). In maize breeding programmes this markers with a polymorphism based on different number of repeated motifs at a given locus are becoming a marker choice (Inghelandt *et al.,* 2018, Kumari *et al.,* 2018).

This marker became the choice for fingerprinting purposes in many plant species due to their high polymorphism, co dominancy and reproducibility and stability of results and in addition to their usefulness in mapping and breeding (Gupta and Varshney, 2000).

Because of the high variability in the number of repeat units it is widely used in many areas of crop improvement. SSR markers are useful tool to explore the molecular diversity among the maize genotypes, as they are not influenced by the environment which can help breeders for selection of diverse parental line which is useful for hybridization programme in heterosis breeding for maize yield improvement.

It can be used for mapping locations within the genome specifically in genetic linkage analysis to locate a gene or a mutation responsible for a given trait or disease. It can also be used for studies of gene duplication or deletion.

Materials and Methods

0.5gm of leaf sample was cut into small bits with the help of sterile scissors and grinded with 2ml of hot (65°C) CTAB extraction buffer in mortar and pestle. Then transfer 600µl of extract into eppendorf tube. Incubate the tube on water bath at 65°C for 45 minutes with occasional mixing. After 45 minutes the tubes were removed from the water bath and equal volume of Chloroform : Isoamyl alcohol mixture (24:1 v/v) was added and mix gently by inversion.

Then centrifuge at 12,000 rpm for 5min at room temperature. The clear aqueous phase was transferred to a new eppendorf tube. Add $2/3^{rd}$ volume of chilled isopropanol and mix gently by inversion and incubate on ice bath for 30 minutes.

Then it was centrifuge at 12,000 rpm for 15 minutes at room temperature. Supernatant was discarded and DNA pellet was washed with 70per cent ethanol (200µl). Then centrifuge at 12,000 rpm for 10 minutes at room temperature. The alcohol was decanted and DNA pellet was air dried. Then lastly, DNA pellet was dissolved in 30µl TE buffer (0.1) and stored at -20°C.

DNA was diluted and subjected to PCR amplification by using the 22 SSR primers (Table 1). Firstly the cocktail were prepared in the pcr tube using 2.0µl of template DNA, 5.9µl milliQ water, 1µl of PCR buffer with 1.5mM $MgCl_2$, 0.2µl of $dNTP_s$, 0.2µL of Forward primer and 0.2µl of reverse primer, 0.5µl of Taq DNA polymerase for PCR amplification.

The reaction mixture was short spin then the PCR tubes were loaded on automated thermal cycler. The steps used in PCR reaction were as follows:

94°C for 5 min
94°C for 1min
53-48°C for 30 sec
72°C for 40 sec
94°C for 1 min
48°C for 30 sec
72°C for 45 sec
72°C for 5 min

Data analysis and scoring

The amplified bands were scored on the basis of presence or absence of bands where presence of bands were donated by 1 and absence were denoted by 0. The PIC (Polymorphic information was calculated using Power Marker 3.5. Coefficients of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by agglomerative technique using the UPGMA (Unweighted Pair Group Method with Arithmetic mean) method by SAHN clustering function of NTSYS-pc version 2.02 (Rohlf, 1994).

Results and Discussion

Analysis of all the 14 SSR primer which show polymorphism across the 10 inbred lines. A total no. of 53 alleles was amplified. The PIC value ranged from 0.46 (Phi046) to 0.81(Phi059) with an average of 0.76.

The SSR analysis showed maximum genetic similarity between CLQRCY 44 and DHM 117 (0.65) and minimum genetic similarity between CLO2450 and BML-6 (0.03) (Fig.1–7 and Table 2).

Table.1 List of genotypes taken for the study

Serial no.	Genotype
1	LM-14
2	CLO2450
3	SML-1
4	LM-13
5	CML-451
6	BML-6
7	BML-7
8	CLQRCY-44
9	DHM-117
10	PAC-740

Table.2 List of primers and their PIC value

S.No.	Primer code	Freq.	PIC value
1.	Phi 006 Phi006 Phi006	0.04 0.04 0.02	0.64
2.	Phi059 Phi059 Phi059	0.03 0.03 0.03	**0.81**
3.	Phi063 Phi063	0.06 0.04	0.48
4.	Phi062 Phi062 Phi062	0.04 0.05 0.01	0.58

5.	Phi065 Phi065 Phi065	0.02 0.04 0.04	0.64
6.	Phi050 Phi050 Phi050	0.03 0.02 0.05	0.62
7.	Nc130 Nc130 Nc130 Nc130	0.02 0.02 0.03 0.03	0.74
8.	Phi032 Phi032 Phi032 Phi032 Phi032	0.03 0.02 0.01 0.02 0.02	0.78
9.	Phi064 Phi064 Phi064 Phi064	0.04 0.04 0.02 0.02	0.72
10.	Phi034 Phi034 Phi034	0.03 0.02 0.05	0.62
11.	Phi014 Phi014 phi014	0.03 0.04 0.03	0.66
12.	Phi073 Phi073 Phi073	0.03 0.03 0.04	0.66
13.	Phi011 Phi011	0.04 0.06	0.48
14.	Phi053 Phi053	0.04 0.06	0.48
15.	Phi024 Phi024 Phi024	0.02 0.02 0.06	0.56
16.	Bnlg391 Bnlg391 Bnlg391 Bnlg391	0.02 0.01 0.03 0.04	0.70
17.	phi046 Phi046 phi046	0.07 0.02 0.01	0.46

	LM14	CLO2450	SML1	LM13	CML451	BML6	BML7	CLQRCY44	DHM117	PAC740
LM14	1	0.269	0.148	0.214	0.138	0.129	0.062	0.097	0.1	0.062
CLO2450		1	0.2	0.138	0.185	0.03	0.1	0.065	0.067	0.138
SML1			1	0.107	0.111	0.067	0.148	0.148	0.154	0.069
LM13				1	0.222	0.207	0.172	0.133	0.065	0.097
CML451					1	0.133	0.222	0.138	0.067	0.222
BML6						1	0.4	0.346	0.308	0.167
BML7							1	0.545	0.375	0.214
CLQRCY44								1	0.65	0.308
DHM117									1	0.435
PAC740										1

Fig.1 Similarity matrix for Jaccard's coefficient for 10 maize genotypes based on SSR analysis

The UPGMA based dendrogram of 10 inbreds lines separated into two well defined groups.

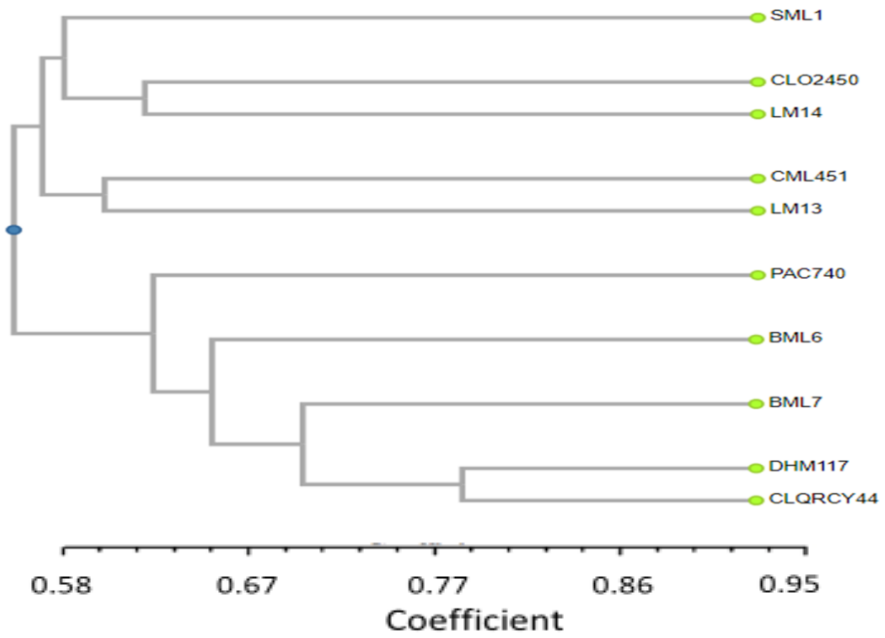

Fig.2 UPGMA based cluster analysis of 10 maize inbred lines using SSR markers.
It was supported by Patel *et al.*, (2017)

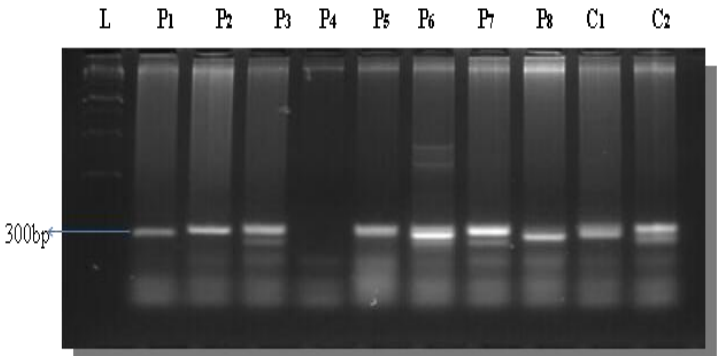

Fig.3 Phi006 SSR marker

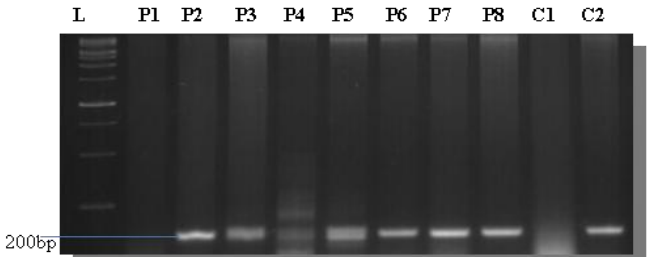

Fig.4 Phi059 SSR marker

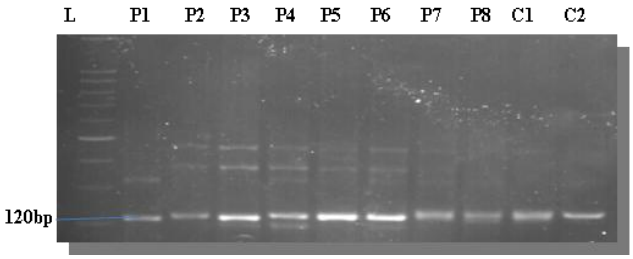

Fig.5 Phi062 SSR Marker

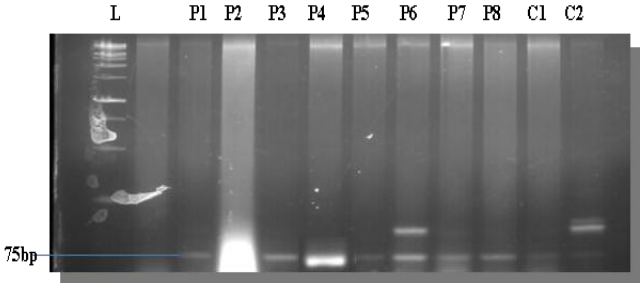

Fig.6 Phi063 SSR marker

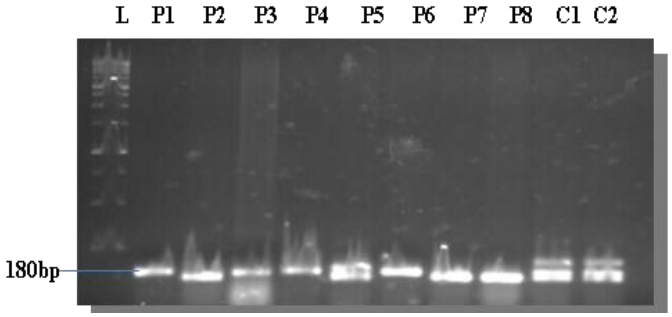

Fig.7 Phi065 SSR marker

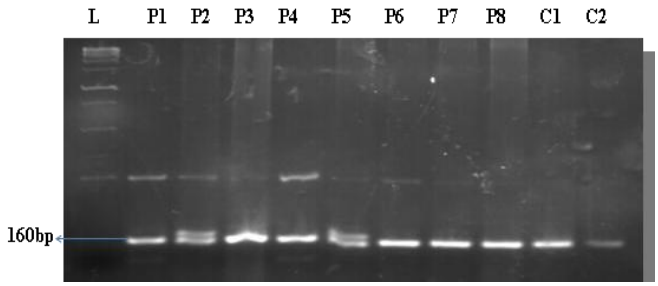

Fig.8 Phi050 SSR marker

SSR markers have been increasingly used as efficient tools to determine genetic diversity and relationship among maize inbred lines. In this study, PIC value indicated a good efficiency of markers for studying the polymorphism level available in studied inbred lines. High level of diversity among the inbreds detected with SSR markers indicated their suitability for further breeding programs.

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