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Genetic Analysis of Some Commonly Grown Genotypes of Maize in Pakistan Using Maize Specific Simple Sequence Repeat (SSR) Primers

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Abstract: Maize (*Zea mays*) belongs to the family gramineae (poaceae), genus *Zea* and species *mays*. The genome of maize comprises approximately 2500 million base pairs. During the present study, maize specific Simple Sequence Repeat (SSR) primers were used to estimate genetic distances among fourteen varieties/genotypes of maize commonly cultivated in Pakistan. On an average, 3.2 alleles per genotype were amplified. Average genetic distance estimated using the four SSR primer sets ranged from 0 - 53%. Dendogram based on DNA data showed that the fourteen genotypes were clustered in six groups A, B, C, D, E and F comprising 3, 4, 2, 2, 2 and 1 genotypes respectively. It has been concluded that genotypes 6098 and Ev- 1097 were most distantly related to each other.

Key words: Dendrogram, genetic diversity, PCR, Phylogenetic elaboration, simple sequence repeats, *Zea mays*

INTRODUCTION

Maize (*Zea mays*) is an important cereal crop after rice and wheat in the world. Maize belongs to family gramineae (poaceae) and genus *Zea*. Morphologically maize is classified according to its colour; Yellow maize, White maize, Red maize and Maize of no definite colour. It is widely cultivated in the tropics, sub tropics and temperate regions of the world including Africa, America, and Asia (Morris, 1998). In the world, maize is cultivated on the area of 143,331,335 ha with a total production of 637,444,480 metric tones giving an average yield of 3.41 metric tone per ha (Anonymous, 2007). The largest producer and exporter of maize is United State of America and leads production with 256,904,560 metric tons from an area of 28,789,240 ha. In Pakistan, maize is grown in summer (kharif) season. The total area under maize cultivation is 981,800 ha with total production of 1,275,000 metric tones, which gives an average yield of 1.46 metric tones per ha (Anonymous, 2006).

Maize has a large genome (2365 Mb) and consists of 60-80% repetitive sequence (Yuan *et al*., 2003; Messing *et al*., 2004). In the past not much work has been done in Pakistan, for maize improvement, using molecular techniques. Present study was aimed at characterization of local germpalsm of maize using maize specific Simple Sequence Repeat (SSR) primers (Anonymous, 2004). Genetic distances among 14 maize genotypes were estimated and Phylogenetic elaboration of maize genotypes was carried out using cluster analysis.

MATERIALS AND METHODS

During present study DNA of 14 genotypes of *Zea mays* (obtained from CCRI, Pir Sabak and National Agricultural Research Centre, Islamabad) were analyzed using maize specific Simple Sequence Repeats (SSR) primers. Plants were planted at the Department of Genetics, Hazara University, Mansehra during summer 2009. A small scale DNA isolation procedure developed by Weining and Langridge (1991) was used with minor modifications to isolate total genomic DNA from the fresh leaves. Quality and quantity of the DNA was checked on 1% agarose/TBE gel. Four maize specific Simple Sequence Repeat (SSR) primer sets used during present study were kindly provided by Dr. A. Carrera, University of Southern Argentina (Table 1). Polymerase Chain Reaction (PCR) was carried using protocol described by Mukhtar *et al*. (2003) and Devos and Gale (1992). The PCR Products were electrophoretically separated on 2% agarose gels and visualized after staining with Ethedium bromide using Uvitech gel documentation system.

For statistical analysis each individual band was considered as a single locus/allele. Alleles/loci (bands) were scored as present (1) or absent (0). Genetic diversity was estimated using following formula (Nei and Li, 1979).

$$GD = 1 - d_{xy}/d_x + d_y - d_{xy}$$

where; GD = Genetic distance between two genotypes,
d_{xy} = number of common bands in 2 genotypes,

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Table 1: Sequence of SSR primers used during present study

S.No	Oligo name	Sequence (5'-3')
1	p-umc1354	GATCAGCCCGTTCAGCAAGTT (F)
		GAGTGGAGGCGGAGGATCTG (R)
2	p-umc1472	TTTTTCTTCTCACCATCACCTTCA (F)
		TGGCTTCAAAGAAGAGGAAACATC (R)
3	p-bnlg1025	TGGTGAAGGGGAAGATGAAG (F)
		CCGAGACGTGACTCCTAAGC (R)
4	p-umc1715	TTCATTGGGTCTCTAGCCAAGAAG (F)
		GGGGAGTCACAGATCTCATCAACT R)

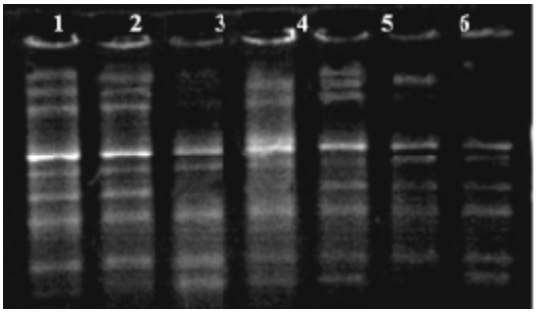

Fig. 1: PCR amplification profile of seven maize genotypes using SSR primer set p-umc1354. 1 = BS 1, 2 = Ev 5098, 3 = Sahiwal 2002, 4 = Ev 1097, 5 = Islamabad yellow, 6 = Pol 2004, 7 = Islamabad white

d_x= total number of bands in genotype 1 and d_y = total number of bands in genotype 2. The bi-variant 1-0 data matrix was also used to construct a dendrogram using computer program "Popgene 3.2" (Yeh *et al*., 1999).

RESULTS AND DISCUSSION

The amplification profile of various genotypes using 4 SSR primer sets showed alleles of different molecular weight. An example of PCR amplification of 7 genotypes using SSR primer set p-umc1354 is presented in Fig. 1.

Average genetic distance estimates (GD) using 4 SSR primer sets are presented in Table 2. Based on 4 SSR primer sets, average GD among the genotypes ranged from 0% (among Sadaf and BS2) to 53% (among EV2004 and Islamabad yellow). Dendogram based on 4 SSR primer sets is presents in Fig. 2. Fourteen genotypes were clustered in 6 groups (A-F), comprising 3, 4, 2, 2, 2 and 1 genotypes, respectively. Group A comprised three

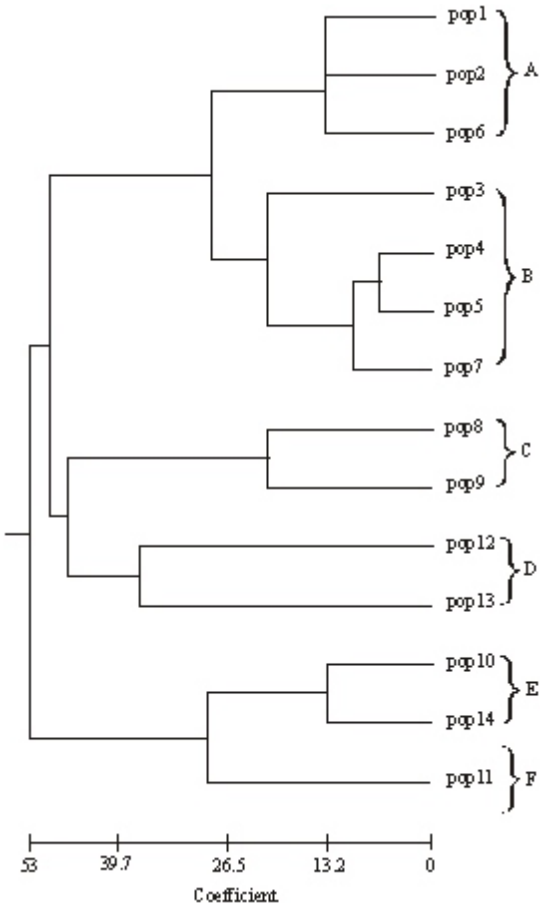

Fig. 2: Dendogram constructed for 14 genotypes of maize using 4 SSR primer sets. Pop 1 = 6098, pop 2 = Soan 3, pop 3 =Agaiti 2000, pop 4 = Ev 2004, pop 5 = Sadaf, pop 6 = BS2, pop 7 = Pak Afgy, pop 8 = BS 1, pop 9 = Ev 5098, pop 10 = Sahiwal 2002, pop 11 = Ev 1097, pop 12 = Islamabad yellow, pop 13 = Pol 2004, pop 14 = Islamabad white. A-F = phylogenetic groups

Table 2: Average estimates of genetic distances among 14 genotypes of maize using 4 SSR primer sets

	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.12												
3	0.33	0.33											
4	0.25	0.25	0.17										
5	0.08	0.08	0.33	0.17									
6	0.17	0.17	0.42	0.25	0.00								
7	0.22	0.08	0.33	0.33	0.08	0.08							
8	0.19	0.31	0.46	0.33	0.17	0.42	0.25						
9	0.35	0.35	0.50	0.33	0.17	0.25	0.25	0.25					
10	0.40	0.40	0.46	0.37	0.21	0.43	0.28	0.37	0.28				
11	0.29	0.12	0.40	0.37	0.21	0.18	0.25	0.28	0.28	0.18			
12	0.34	0.37	0.45	0.53	0.36	0.17	0.23	0.36	0.26	0.40	0.30		
13	0.33	0.47	0.28	0.22	0.39	0.33	0.46	0.36	0.36	0.48	0.40	0.47	
14	0.25	0.25	0.50	0.33	0.17	0.25	0.23	0.23	0.23	0.06	0.21	0.36	0.42

1 = 6098, 2 = Soan 3, 3 = Agaiti 2000, 4 = Ev 2004, 5 = Sadaf, 6 = BS2, 7 = Pak Afgy, 8 = BS 1, 9 = Ev 5098, 10 = Sahiwal 2002, 11 = Ev 1097, 12 = Islamabad yellow, 13 = Pol 2004, 14 = Islamabad white

genotypes viz; 6098, Soan 3 and BS2. Group B comprised Agaiti 2000, Ev 2004, Sadaf and Pak afgy. Group C consisted Bs 1 and Ev 5098. Group D comprised Islamabad yellow and pol 2004. Group E composed of Sahiwal 2002 and Islamabad white. While group F consisted Ev 1097.

CONCLUSION

It is concluded that PCR using species specific Simple Sequence Repeat Primers (SSR) can reliably be used for the estimation of genetic diversity in crops of commercial importance like maize. On the basis of dendrogram analysis, it has been concluded that genotypes 6098 and EV 1097 were most distantly related to each other. It is suggested that breeding program aimed at increasing genetic variability within maize genotypes should be launched and those programs may involve hybridization among these diverse genotypes. It is also suggested that more studies of similar nature should be conducted for better understanding of the genome structure of maize genotypes, which will ultimately help designing better strategies for maize crop improvement.

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