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Molecular diversity of babycorn (*Zea mays*) inbred lines by rice SSR Marker

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Baby corn (*Zea mays* L.) is one of the speciality corns which have quite comparable nutritive value with other vegetable crop. It is the immature, unfertilized cob of maize. DNA based molecular markers such as SSR provide favourable tool for genetic analysis. Simple Sequence Repeats (SSRs) are short and tandem repeats of few base pairs (1-6 bp) with abundant exons, introns ,5'-UTRs,3'-UTRs (Avinash *et al*. 2014) which are categorised in genomic SSR which can be used to determine rapport among individual of same as well as different species of same family, their fingerprinting and phylogenetic study. Development of SSR or, microsatellite is highly expensive. Only up to 30% of all primers that were developed from sequences are functional and provide desirable outcome for genetic analysis. Increase in cost creates limiting factor in research area for more exposure to genomic information (Avinash *et al*. 2014). It is reported that 80-90% of maize genome is similar to rice genome as they belong to same family *Poaceae* (Anne *et al.* 2009). Thus, by identification of comparative mapping study one can clearly disclose the presence of synteny within the genomes of the closely related species of grass family. There is sufficient homology present in between several crop genomes in the sequences that are flanking the SSR loci (Chin *et al*.1996) SSR markers proved to be best, highly abundant and polymorphic as it has more reproducibility, it is multiallelic and co-dominant in nature and it has good genome coverage. So, it can be used for the study of genetic diversity of related species that have been taken. Keeping this view in mind, present study was done to study the similarity between rice and maize genome by using rice SSR markers.

Plant materials and DNA isolation: A set of 24 maize genotypes (Table 1) belonging to different genetic background as well as locations were collected from Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour, Bhagalpur (2018). The genomic DNA was extracted from 10-15 days plant's leaf sample by the modified DELLAPORTA METHOD (Dellaporta *et al*. 1983) the quality and quantity of extracted DNA was checked by Agarose Gel Electrophoresis in 0.8%. After checking, DNA sample was diluted at the concentration 40 ng/µl for SSR analysis. The PCR amplification was done in 10 µl reaction volume containing 1 × PCR buffer, 0.2 mM dNTPs, 0.2 µM each forward and reverse primer, 0.5 U Taq DNA Polymerase (Xcelris, India) and 40 ng of template DNA using a thermal cycler (Eppendorf, Germany). Touchdown-PCR reactions were performed as follows: 4 min pre-denaturation at 94°C, followed by 94°C for 30 sec, 60°C for 40 sec, and 72°C for 40 sec in the first cycle, then decreasing the annealing temperature by 1°C/cycle for 10 cycles, followed by 94°C for 30 sec, 55°C for 40 sec, and 72°C for 40 sec for 25 cycles and ending with 5 min of elongation at 72°C. The amplified PCR products along with 100 bp DNA ladder as molecular marker were resolved in 2% agarose gel stained with ethidium bromide (0.5 µg/ml). Gel was visualized under UV light and documented in gel documentation system (Uvitec gel doc system, UK).

Table 1 List of genotypes taken for the study

Genotype	Genotype
CLQRCY 44	VL 1055
SML 1	VL 145312
LM 14	VQL 1
LM 13	HKI 1105
BML 7	DHOLI 55
CML 451	95 IOWA
CLO 2450	96 ROHYO
BML 6	SUWAN
CM 400	CM 600
CM 501	HKI 335
ZL 14501	HKI 1532
VL 1010762	CML 425

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Table 2 List of primers shown polymorphism

Primer code	Sequence (5'-3')	Chr. No.	PIC value	No.of Polymorphic alleles
RM566-F	ACCCAACTACGATCAGCTCG	9	0.28	4
RM566-R	CTCCAGGAACACGCTCTTTC			
RM1009 –F	GATGCTCCGGAATAACTAGATTGG	1	0.34	3
RM1009-R	GGAATTACAGCTGTCTTGGAAGG			
RM28099-F	TGTGCGGATGCGGGTAAGTCC	12	0.28	4
RM28099-R	CCACCTGTCAACCACCGAAACC			
RM523-F	AAGGCATTGCAGCTAGAAGC	3	0.25	4
RM523-R	GCACTTGGGAGGTTTGCTAG			
RM555-F	TTGACATGCGAAATGGAGATGG	2	0.3	4
RM555-R	TTGGATCAGCCAAAGGAGACC			
RM16030-F	GCGAACTATGAGCATGCCAACC	3	0.32	4
RM16030-R	GGATTACCTGGTGTGTGCAGTGTCC			
RM11943-F	CTTGTTCGAGGACGAAGATAGGG	1	0.26	4
RM11943-R	CCAGTTTACCAGGGTCGAAACC			
RM511-F	CTTCGATCCGGTGACGAC	12	0.22	4
RM511-R	AACGAAAGCGAAGCTGTCTC			
RM216-F	GCATGGCCGATGGTAAAG	10	0.14	
RM216-R	TGTATAAAACCACACGGCCA			
RM552-F	CGCAGTTGTGGATTTCAGTG	11	0.36	1
RM552-R	TGCTCAACGTTTGACTGTCC			

Data analysis and scoring: The molecular analysis depicts an array of characters which were converted into binary system (Sneath and Sokal 1973) based on presence of amplification of band for each allele. The presence of clear and distinct band was scored as giving '1' and '0' to absence of band corresponding among all 24 genotypes. The polymorphic information content (PIC) values for each primer were calculated using Power Marker 3.5. Similarity matrix was generated using the SIMQUAL function of NTSYS-pc version 2.02 (Rohlf 1998). The Jaccard's similarity coefficients were used for cluster analysis and Phylogenetic tree or, Dendrogram was constructed using the Unweighted Pair-Group method (UPGMA) by SAHN clustering function of NTSYS software.

The study was undertaken mainly to screen out the rice SSR markers on maize genotypes. Among forty Rice SSR markers, fourteen SSR markers (RM566, RM1009, RM28099, RM493, RM492, RM523, RM555, RM16030, RM11943, RM511, RM216, RM5791, RM219, RM552) showed amplification in maize genome. Out of 14, 10 SSR markers showed polymorphism among different genotypes for the study of genetic diversity in maize. Rice SSR markers that have shown polymorphism in maize genotypes is listed in Table 2.

The polymorphic information content (PIC) value was calculated and maximum value was 0.36 and minimum value was 0.14 with average of 0.28. Marker RM552 showed highest PIC value followed by RM 1009 and RM 16030. The lowest PIC value was from primer RM 216. By the Jaccard's similarity coefficient it ranged from 0.05- 0.65. The dendrogram construction was done by using NTSYS-software by UPGMA method and formed four clusters in which 24 genotypes were grouped (Fig 1). The Genotype CML425 revealed to be the most diverse genotype. Among all that CML425 reflected its unique genetic structure.

The present study identifies the similarity between the genomic sequence of rice and maize of same family poaceae by using SSR markers. The emergence of DNA based marker technology has provided the rapid generation of many genetic and physical map using and identifying common markers that can also be used for diversity study of concerned crop (Avinash *et al.* 2014). In the present study, 14 SSR markers have shown amplification out of which 10 SSR markers exhibited polymorphism and rest showed monomorphism and did not show amplification in some of the genotypes. The present finding was supported by Ranatunga *et al*. (2004), Avinash *et al*. (2014), Gupta *et al*. (2016) and Kumar *et al*. (2016). Similar results were found by Babu (2017) and Kumari (2018) by using maize SSR in fingermillet genotypes in which 24 markers exhibited polymorphism with PIC value 0.14-0.69. Selvi *et al.* (2003) studied cost effective and potential marker identification of maize SSR in sugarcane genome.

The study indicated the existence of molecular diversity and interrelationship with genetic structure of maize genotype and rice genome from where these SSRs developed. The highest Polymorphic information content (PIC) value indicated by primer RM552, i.e. 0.36 that signifies its potential to study genetic diversity across other crops like rice. This can be used as easy and cost effective

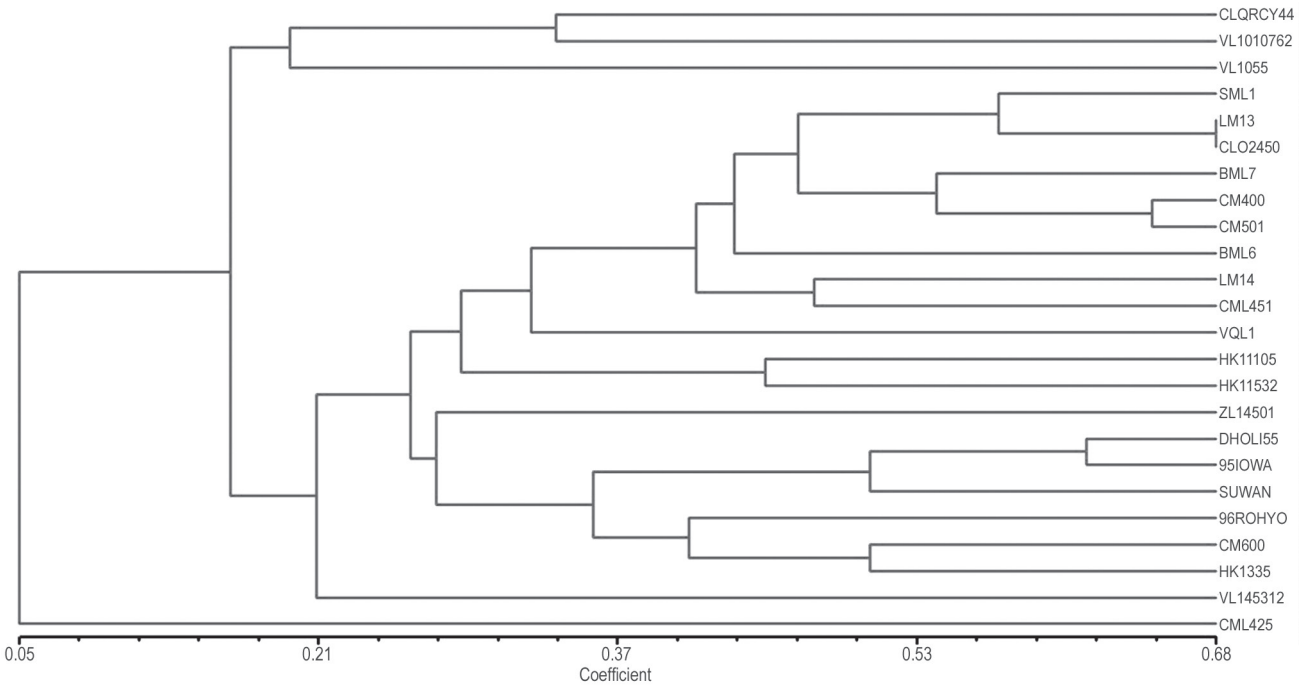

Fig 1 Dendrogram showing clustering of 24 maize genotypes based on SSR marker analysis.

approach to validate such markers as common markers for diversity study of different crop species.

SUMMARY

A set of 24 maize inbred lines were analysed by using Rice SSR markers (40). Out of which, 14 SSR markers were found to be amplified and reproducible in which 10 markers showed polymorphism and rest showed monomorphic bands. A total of 32 polymorphic alleles were found. The average polymorphic information content (PIC) value was 0.28 with maximum 0.36 (RM552) and minimum 0.14 (RM216). By the use of Unweighted paired group method (UPGMA) for cluster analysis and formed four clusters in which genotype CML425 was found extremely diverse from rest of the genotypes. This represents its unique genetic structure from the rest genotypes. The overall result indicates that the genotypes present in these clusters have maximum genetic diversity that will help in classifying various genotypes in exact heterotic groups for future breeding programme. The study provided sufficient information about the genetic diversity of maize inbreds and that also identified common markers for rice and maize genetic studies that can be helpful for reducing cost effective measures for producing crop specific markers. In this way, identified clusters may be used for future breeding programme. This study also concluded that SSR marker can be taken as good complementing tool.

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