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Genome analysis of sugarcane (cultivar BL4) to investigate transposable elements

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Abstract: Transposable elements (TEs) are abundant in eukaryotic genomes. About 50-80% of the genomic DNA of grasses is represented by TEs and these elements are responsible for the genome size variations. TEs are considered to have potential involvement in the phenotypic evolution. This study relied on degenerate primers for detection of Ty1-copia elements (class I TE or retroelement) and designed primers from the conserved sequences for the detection of Mutator (*Mu*) and Activator (*Ac*) (class II TEs) elements. The results obtained through PCR amplification confirmed that all three types of TEs investigated were present in BL4.

Keywords: sugarcane, transposable elements, Ty1-copia, mutator (*Mu*), activator (*Ac*)
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INTRODUCTION

Transposable elements (TEs), the genome wide dispersed repetitive sequences are thought to be the major cause of the genomic instability because of their ability to move within the genome. TEs are abundant in eukaryotic genomes and 50-80% of the genomic DNA of grasses is composed of TEs[1-3]. The amount of repeated DNA sequences influence the genome size, and the genome size fluctuations play a role in the selection of new phenotypes.[4,57-9] . Transposable elements (TEs) are classified mechanistically into two major groups: RNA mediated transposable elements (class I) and DNA transposable elements (Class II) that can propagate as DNA with the help of a transposase[10]. Class I transposons also known as Retroelements, transpose via an RNA intermediate which transcribes into cDNA with the help of reverse-transcription and integrated elsewhere in the genome. Retrotransposons include subclasses of ***copia*** and ***gypsy*** with long terminal repeats (LTR) on the flanking region and **LINES** (Long interspersed nuclear element) and **SINES** (small interspersed nuclear element) without LTRs[2, 11].

Deoxyribonucleic acid transposons, or class II elements, are characterized by terminal inverted repeats (TIRs) and they encode a transposase (Tpase), the protein responsible for excision and insertion of transposons. Activators (*Ac*), Mutator *(Mu*) mutators,Ac are the class II TEs.

Modern sugarcane cultivars, developed from the inter-specific crosses possess the most complex genome of any crop plant [12]. Polyploidy in *Saccharum* is widespread and has been largely responsible for the genetic and taxonomic complexity of this genus[13]. *Saccharum* poly aneuploid hybrids have chromosome numbers usually in excess of 100. The size of the modern sugarcane genome is 10 Gb/2C[14-15]. The Brazilian Sugarcane EST Sequencing Project (SUCEST) has identified a wide diversity of transcriptionally active TEs in the sugarcane genome.

In the present study, the degenerate primers developed by Flavell et al.[16] were used for the identification of Ty1- copia elements (class I TEs) in sugarcane variety BL4, a local (Sind, Pakistan) cultivar of sugarcane. More primers were designed from the conserved sequences of mutator and Activators like TEs to investigate their presence in BL4.

MATERIALS AND METHODS

DNA extraction

DNA from the young leaves of sugarcane (*Saccharum spp.* hybrid) variety BL4 was extracted as described by Puchooa et al.[17] with some modifications. The quantity and quality of DNA was checked by Spectrophotometer at 260nm and 280nm.

DNA transposable elements (Class I): Ty1-copia elements

Two sets of Degenerate primers developed by Flavell[16] et al. and Hirochika & Hirochika[18] were used for the identification of Ty1-copia elements in sugarcane variety BL4. The sequences of individual primers were as noted below:

Forward Primer 1: CARATGGARGTNAARAC
Reverse Primer1: CATRTCRTCNACRTA
Forward Primer2: ACNGCNTTYYTNCAYGG
Reverse Primer2: ARCATRTCRTCNACRTA
Where R= A or G=purine bases; N=A,G,C or T=any ; Y=C or T=pyrimidine

PCR reaction was carried out in 10µl reaction mixture containing 100ng of template (genomic DNA), 1.5mM MgCl2, 0.2mM of each dNTPs, 1.5U of Taq polymerase and 0.6µM of primer in a 1xPCR reaction buffer. The amplification reaction was performed in the Eppendorf Master cycler with an

initial denaturation for 4 min at 94°C, then 35 cycles: 30sec denaturation at 94C; 35sec annealing at 39°C; 30sec extension at 72°C. Final extension was carried out at 72°C for 7 min. Amplified products were analyzed through electrophoresis on 1.2% agarose gel containing 0.5X TBE (Tris Borate EDTA) at 60 Volts for 1.5 hrs, photograph was taken under UV light using gel documentation system.

***DNA transposable elements (Class II)*: Mutator (Mu) like TEs**

Alignment of all seven expressed sequence tags (ESTs) for mutator like transposable elements (Gene Bank accession gi76261912, gi 76261905, gi76261946, gi76261920, gi76261909, gi76261914, gi76261915, gi76261911 was done by CLUSTALX and viewed on GeneDoc to determine the conserved sequences. Subsequently, primers were designed from 820bp long conserved sequence of mutator homologs from the Primer 3 software. Three sets of primers were used to detect the mutator like elements in the local cultivar BL4:

mutF1:GCATGTGTCTGGGATTCCTT,

mutR1:AGCGCACTGCTCTTTTGATT;

mutF2:CTGGGATTCCTTGCTCACAT,

mutR2:AGCGCACTGCTCTTTTGATT;

mutF3:TCACGACTTGGCCAGTACAC,

mutR3 AAGGAATCCCAGACACATGC.

All PCR conditions were same as for Ty1-copia element detection except annealing temperature which was 55°C.

DNA transposable elements (Class II): Activator (Ac) like TEs

Alignment of all ESTs for activator like TEs (GeneBank accession No. gi76261907, gi76261897, gi76261882 was done by CLUSTALX. Subsequently, primers were designed using Primer 3 software from 383bp long conserved sequence of activators homologs. The sequences of primers were

AcF1 GAGATTCCCAGTGCCAAGAC,

AcR1 CGGACTTTCATGTTCTGTGC;

AcF2 CAGTGCCAAGACCCTTTGTT,

AcR2 TGAATTTTCCAAGCTTCATGG.

All PCR conditions were same as for Mutators and Ty1-copia detection except annealing temperature which was 53°C.

RESULTS AND DISCUSSION

PCR for Ty1-copia elements produced 270bp long amplicon (Figure 3 A) using the 2nd set of primers, developed by Flavell[16] et al. proves that the Ty1-copia, retroelements are present in sugarcane. This set of primers was used in a variety of angiosperms with diverse sequence polymorphism by Flavell et al[16]. and rarely failed to get the desired product. However, the primers designed by Hirochika and Hirochika[18] did not amplify the desired product, may be due to the sequence variations in the primer binding sites.

A number of partial sequences ranging from 0.64 - 2.3 kb of Mutator and 0.37-2.kb of activator like TEs has been deposited to the genebank by Araujo et al[1] isolated from different clones of *Saccharum* hybrid cultivar SP80-3280. For mutator identification, primers were designed from the 820 bp long most conserved sequence (Figure 1), however it was observed that gi76261912, gi 76261905, gi76261946, gi76261920 had more consenus regions than other sequences. In order to amplify mutators that do not fall in the conserved catagory, three sets of primers were designed from different parts of the alligned sequence. From the clone BL4, amplified products of 284, 276 and 349 bp respectively (Figure 3B) were identified. Two sets of primers from the 380bp long conserved sequence (Figure 2) were designed to detect the Activator like element. As a result, PCR products of 301 and 309 bp long were obtained respectively (Figure 3C).

The results show that all three types of TEs (Ty1-Copia, Mutators and Activators) investigated in this study were present in the studied cultivar BL4. Further sequencing of these PCR products may provide information about the subtypes of copia, mutator and activator like TEs in sugarcane. In addition to this, the designed primer could also be used for the insertional polymorphism of TEs in sugar cane. That could be a good molecular marker for DNA finger printing of sugarcane as used by Huang et al [19].in rice; by Chang et al[20]. in barley; by Nair el al.[21] in banana cultivars.

Figure 1: Conserved sequences of mutator like elements in sugarcane

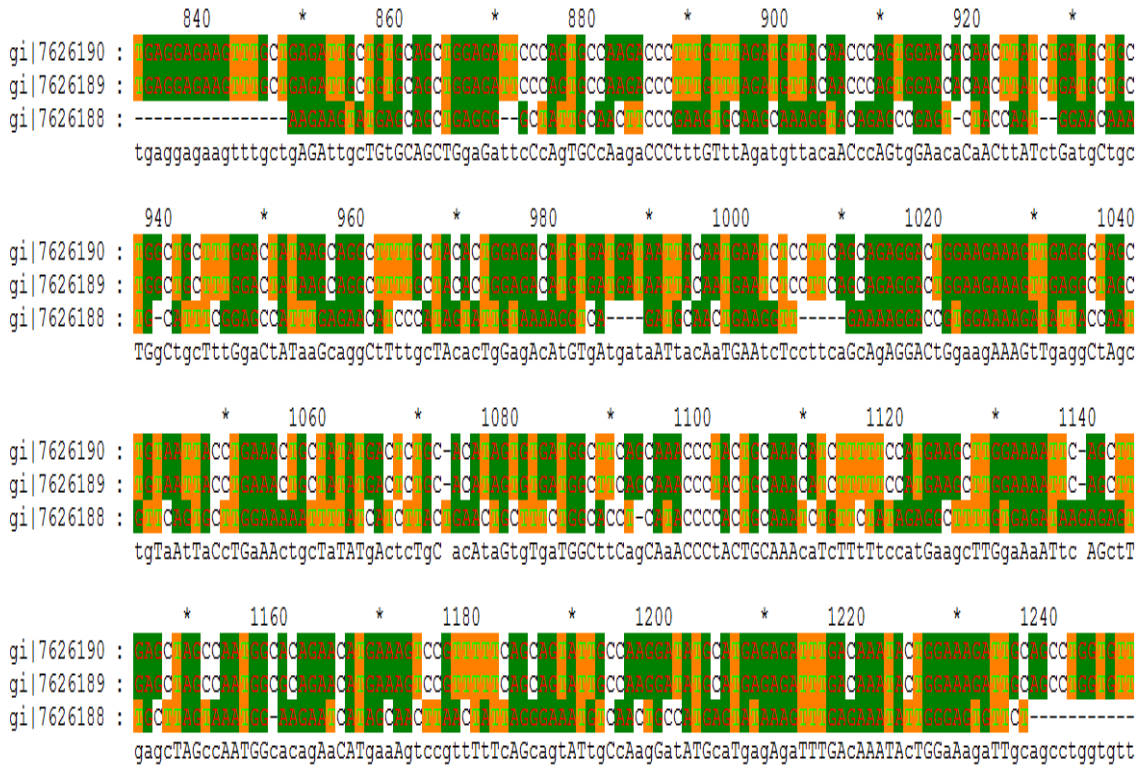

Figure 2: Conserved sequences of activator like elements

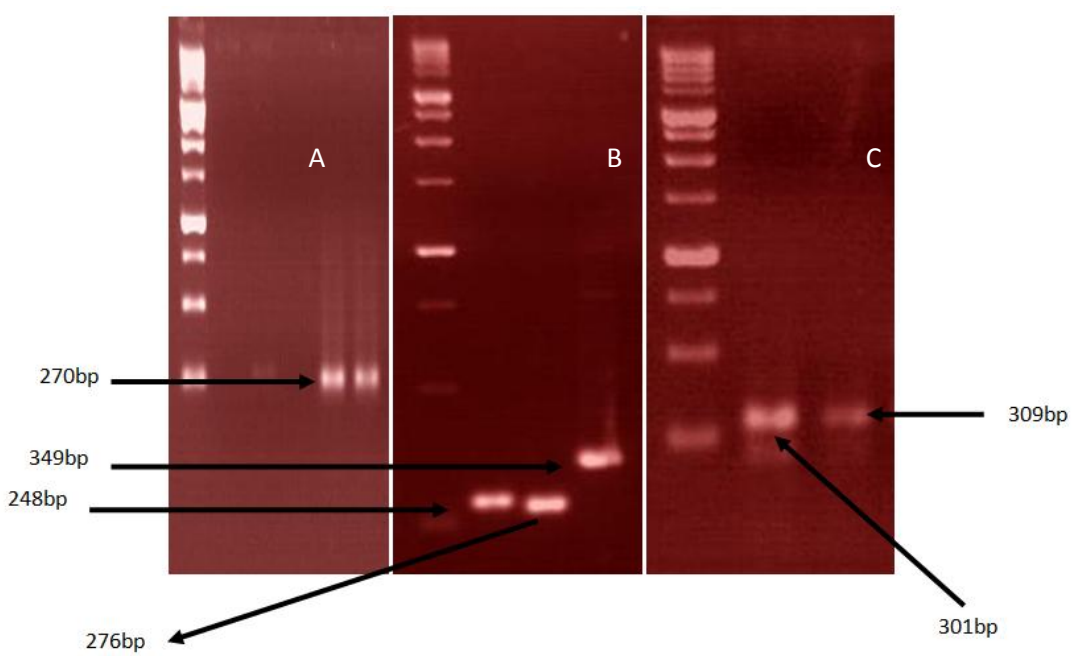

Figure 3: (A) amplified product of Ty1-copia (B) Amplified product of Mutator like TE using three different primers.(C)Amplified product of Activator like TEs using two different primer.

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