Pakistan J. Zool., vol. 51(3), pp 1199-1202, 2019. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2019.51.3.sc6

Short Communication

Diversity of Retrotransposons (Ty3-*gypsy*, LINEs and Ty1-*copia*) in *Sordaria fimicola*

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ABSTRACT

Retrotransposons have become an inevitable source of genetic diversity due to their abundance in eukaryotic genomes and their insertion in coding regions leads to altered gene expression. We examined the diversity of retroelements in four consecutive generations of different strains of *Sordaria fimicola.* The parental strains were collected from two contrasting environments of Evolution Canyon *i.e.* harsh South facing slope and neutral North facing slope. Retroelements profiles were generated in order to detect retrotransposons. Strains from South facing slope exhibited more copy number of Ty3-gypsy, LINEs and Ty1-*copia* as compared to strains from North facing slope. In total, 52 fragments of Metaviridae (Ty3-*gypsy*), 104 fragments of LINEs and 66 fragments of Ty1-*copia* were sequenced. In this way, strains from the contrasting environments were successfully discriminated on the basis of retrotransposons.

Article Information
Received 20 January 2017
Revised 11 July 2018
Accepted 24 December 2018
Available online 24 April 2019

Authors' Contributions
MS and RA designed the research. TJ and SI performed the experimental work. FA helped in manuscript write up.

Key words
Diversity, Analysis, *Sordaria fimicola*, Transposable elements, Strains.

Retrotransposons can act as molecular markers because they have conserved sequences and their replication leads to polymorphism in genomes. This quality can be used to detect phylogenetic relationships. Genome of an organism either eukaryote or prokaryote is not stationary; many ideas concerning the mobility of genome and their function are put forth (Slotkin *et al*., 2007). Barbara Mccklintock's experiment in 1940 regarding the corn cornel color revolutionized the science of mobile elements (Creighton and Barbara, 1931). Transposons are discrete elements that can move within and between the genome of an organism. Widely, transposons are classified into two categories, DNA transposons and Retrotransposons (Kass and Chomat, 2009). DNA transposons are transposed directly instead of transposition by reverse transcriptase; do not produce RNA copy as intermediary. DNA transposons include P element of *Drosophila*, and *Tc1* element of *Caenorhabditis elegans* and corn AC/DC elements. Retrotransposon are transposable elements that transpose by producing a copy of target segment via retro transcriptase (Cordaux and Batraz, 2009).

Reterotransposans are unique, ubiquitous elements in eukaryotic genome; often constitute more than half of genome in plants. Retrotransposons comprise about 50% of human genome (Amoyyte *et al*., 2014). The kinds and percentage of transposable elements from the same superfamily can vary greatly from species to species, or even from individual to individual. Mobile genetic element, Transposons were extensively studied in genome of bacteria, plants, animals including human but in 1989 transposons based molecular analysis was reported from filamentous fungi (Kempken *et al*., 1998). LINEs (Long interspersed elements) are type of retrotransposons and they are frequently detected in eukaryotic genomes and replicate by copy and paste mechanism. Each element may be thousands of base pair long (Piskurek *et al*., 2009). Ty3/Gypsy elements are closely related to retroviruses. They encode at least four protein domains in the order: protease, reverse transcriptase, ribonuclease H, and integrase (Marín and Lloréns, 2000). The aim of the present study was to amplify and to sequence the various TEs for the discrimination of various strains of *S. fimicola* isolated from two contrasting environment of Evolution Canyon of Mount Carmel, Israel.

Materials and methods

S. fimicola belongs to saprophytic genera and is homothallic; grows well on organic material, isolated from dung of herbivores and is wide spread in nature. It has high mutation rate (Saleem *et al*., 2001). Less is known about its genetic diversity and population's Eco genetic behavior (Kalogeropoulos and Thuriaux, 1985). Stock strains of *S. fimicola* for S1, S2, S3, N5, N6 and N7 were provided by Molecular Genetics Research Lab, University of the Punjab, Lahore, Pakistan. These original fungal strains were collected from the two slopes which are opposing

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0030-9923/2019/0003-1199 $ 9.00/0

each other and situated in the valley in Evolution Canyon. Four generations (F1, F2, F3 and F4) of six parental strains of *S. fimicola* were raised from single spore culture technique and all were maintained and revived on potato dextrose agar media under sterile and controlled environmental conditions. DNA of mature strains was extracted by modified method of Pietro *et al*. (1995). Primers for the amplification of retrotransposons are listed in Table I and regions were amplified using polymerase chain reaction. TE-PCR reactions were done in a volume of 25µl containing 1.5µL DNA, 2.5 µL 10X PCR buffer, 2.5µL $MgCl_2$, 2.5µL dNTPs, 0.5µL each primer (Forward and Backward, Sigma Aldrich), 0.5µL Taq polymerase, 14.5µL DEPC treated water. TE-PCR amplifications were performed in Veriti96 thermo cycler (Applied Biosystem). The optimum annealing temperature was determined for each primer pairs including other several PCR parameters (concentration of DNA, primers, $MgCl_2$ and Taq DNA Polymerase).

The PCR reaction conditions were 94°C for 4 min, followed by 30 cycles of 94°C for 1 min, *copia* 47°C/LINE 44°C for 2 minutes, and 72°C for 1 min, ending with a final extension step of 72°C for 8 min. The amplified DNA fragments (4µL) were mixed with (1µL) of 6X loading dye. The PCR products were resolved electrophoretically on 1.5% Agarose gel at voltage of 100V for 30 min. The gels were stained with ethidium bromide (3µL**)** and photographed under UV illumination. The 100bp DNA ladder was used as a molecular marker to measure the size of amplified DNA band (Invitrogen). The PCR products were purified and sent for sequencing.

Results and discussion

Many TEs are ubiquitous and have been used as molecular markers to analyze genetic diversity. Retrotransposon based genetic analysis was used to dig variations between all the isolates of South and North strains of *Sordaria fimicola* (S1, S2, S3 and N5, N6, N7). Different Primer pairs of retrotransposons were used to amplify the DNA to determine polymorphism. Amplification revealed the unique banding patterns and thus clarified genetic diversity among strains. In total, 52 fragments of Metaviridae (Ty3-*gypsy*), 104 fragments of LINEs and 66 fragments of Ty1-*copia* were sequenced. Among 52 fragments of Metaviridae (Ty3-*gypsy*), 17 were found in NFS strains and 35 in SFS strains (Supplementary Fig. S1). Out of 104 fragments of LINEs, 45 were found in NFS strains and 59 in SFS strains (Supplementary Fig. S2). Out of 66 fragments of Ty1-*copia*, 40 were found in SFS and 26 in NFS (Table II). The differences found in copy number of these three retrotransposons in strains from opposite slopes of 'Evolution Canyon' might be due to the differences in environmental conditions. Sequences of gypsy and LINEs are given in Figure 1.

Table I.- List of primers for the amplification of Transposable elements in different strains of *S. fimicola*.

Primer name	Primer No.	Primer sequence	Annealing temp.
Metaviridae (Ty3-*gypsy*)	Oligo 16 F	5'-MRN ATG TGY GTN GAY TAY MG-3'	47°C
	Oligo 17 R	5'-RCAYTTNSWNARYTTNGCR-3'	
LINEs	Oligo 26 F	5'-RVNRANTTYCGNCCNATHAG-3'	44°C
	Oligo 28 R	5'-GAC ARR GGR TCC CCC TGN CK-3'	
Copia	Oligo 30 F	5'-GCNATGNANGANGAGATGGA-3'	47 ° C
	Oligo 32 R	5'-TGNTCCCAAATCTTTNATCTC-3'	

Ty3-gypsy

GTAACCGTGTCACCCACTGCTCACAGCCCACGCGTAGCTGCGTCATCAGGCTCAACGAGAGGCCGTCGATGTCCAAGGGCTG

GTCTGGACCATGGCTAGTAGGTCTGGCGGCGGTCTCCTTTACATTTTCCTTCCAATTGTCAAGGCTCTCACAGAGCTCGATGA

AACGCAGGTACGGAGATTTGGAGCCTTTGCAGAGTTTGGTGGTTCGCGGGGAAACTTTCAAATCAGGGCTGCCCACTAAGA

LINEs

TTATAAAATTTTTACCCCCCCGCCGGTCCCCCGGGGAGAAACTTTTGGCTGTTCCCGCCGTTTTGAAGGATTNAAAACAGGGCC

TCGTAGAGGGTTTCCAGTGTGTTGTCTTTTGCACCAAAAAGCGGGGGATTCCCCCCCCCACCGCTCGATGGAAAATTTCCCGTT

GAGAGGTGTTGTTTTTTTCNCAAACAAAAAAAAAAGAGTGTCCCCCCCCAC

Fig. 1. Sequence of Ty3-gypsy and LINEs in S1 strain of *S. fimicola.*

Table II.- Total Copy number of transposable element in different strains and subsequent generations of *S. fimicola*.

NFS	Copy No. of Ty3 *Gypsy*	Copy No. of LINEs	Copy No. of Ty1-*copia*	SFS	Copy No. of Ty3 *Gypsy*	Copy No. of LINEs	Copy No of Ty1-*copia*
N7	1	3	3	S1	2	3	3
N7F1	1	4	3	S1F1	2	4	3
N7F2	1	2	3	S1F2	2	4	3
N7F3	1	3	1	S1F3	3	4	3
N7F4	1	4	1	S1F4	3	4	3
N5	1	4	2	S3	2	4	4
N5F1	1	4	2	S3F1	2	4	3
N5F2	1	4	1	S3F2	2	4	3
N5F3	2	4	1	S3F3	3	4	2
N5F4	2	4	1	S3F4	3	4	2
N6	1	3	3	S2	1	4	3
N6F1	1	3	1	S2F1	2	4	3
N6F2	1	3	1	S2F2	2	4	3
N6F3	1	3	1	S2F3	2	4	1
N6F4	1	3	2	S2F4	3	4	1

The results also showed that the genome of comparative strains is closely related to one another. PAST analysis of multivariate showed that the retroelements in South strains are more diverse than the elements of other strains because Southern strains have defensive mechanism that controls the high mutation rate (Saleem *et al*., 2001; Arif *et al*., 2017) and expression of transposons is often measured in relation to mutation. In this work, we aimed to identify potentially active retrotransposons and identified different retrotransposons in the genomes of isolates from different environmental conditions. Similarity of these retrotransposons was found with *Beta vulgaris* and *Glycine max* retrotransposons when analyzed by GyDB (Gypsy Data Base).

In some plants rearrangements of chromosomes can be result of retrotransposons and multiple copies of these elements lead to genetic diversity (Braumann *et al*., 2008). This diversity may increase virulence of pathogen and helps it to evolve, as more severe pathogen (de Jonge *et al*., 2013). Hence active TE near or within the gene change or deactivate gene and results in mutations and subsequent virulence (Hua-Van *et al*., 2011).

Transposable elements tend to insert in genome at two regions *i.e.* near coding region or in the coding region. If transposable elements managed to insert itself near coding region it can greatly influence the model of gene expression and if it is inside coding region it directly alters gene product (Kidwell, 2002). If two same sequences get inserted at different positions they can generate two different types of point mutations and variations on the bases of host DNA sequences and insertion time of both sequences can be calculated by assessing variation at the ends of both sequences which were identical before insertion (Kijima and Innan, 2010).

As many sites of transposable elements are present in *Sordaria* genome specially at south facing slope it can leads to its evolution by homologous recombination at insertion sites its effect can be negligible like inversions or considerable like duplication, translocation and deletion (Zhang *et al*., 2009; Delprat *et al*., 2009).

Conclusion

In conclusion, retrotransposons are the good molecular markers in order to determine genetic diversity. Our experiment amplified three types of retrotransposons in *S. fimicola*. But there was difference in TE abundance in both strains which means that they evolved simultaneously but strain from south facing slope exhibited more polymorphism because of adverse conditions. These findings suggest that occurrence of more TE elements may lead to mutations.

Supplementary material

There is supplementary material associated with this article. Access the material online at: http://dx.doi.org/10.17582/journal.pjz/2019.51.3.sc6

Statement of conflict of interest

The authors declare no conflict of interest.

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