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Full Length Article

Evaluating Short Sequence Repeats (SSRs) Markers in *Sordaria fimicola* by High Resolution Melt Analysis

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Abstract

Sordaria fimicola is found worldwide but little is known about its population structure in any region. To bridge this knowledge gap, we attempted to develop molecular markers to study the genetic variations and population structure in the natural strains of *S. fimicola*. Based on the whole genome sequence of *S. macrospora* we targeted sixteen genomic regions that contain short sequence repeats (SSRs). Primers were then designed to amplify the homologous regions in *S. fimicola*. We used high resolution melt (HRM) analyses and amplicon sequencing to analyse the genetic diversity of the wild strains of *S. fimicola* collected from the opposing slopes (i.e., South facing slope and North facing slope) of the Evolution Canyon (EC), representing contrasting environments. We found twelve regions that were homologous to that of *S. macrospora* containing (CAT)7, (TTG)7, (T)14, (GAA)7, (CGT)6, (AAGCAC)6, (GT)5, (GGC)6 (ACA)8, (CTG)7 (CT)6 and (CAGGGG)5 were first time reported in *S. fimicola*. During this genotyping of SSRs we found variations in SSRs motif number and enrichment in strains isolated from stressed and harsh environment than strains from natural and benign environment in Evolution Canyon. The findings favour the hypothesis that genetic variations are present in strains of stressed environment.

Keywords: Short sequence repeats (SSRs); Evaluation; *Sordaria fimicola;* Evolution canyon; Genetic diversity

Introduction

Evolution Canyon (EC) is a micro scale divergent environment displaying vast biodiversity patterns at Mount Carmel Israel having three landforms: dry conditions from Asia and Africa, on the south-facing slope (SS), and mesic environment from Europe, on the north-facing slope (NS) (Nevo, 1995; 1997; 2001). EC has distinct inorganic and organic interslope differences that make it appropriate to study evolution, genetic diversity and for ecological testing. At EC the south-facing slopes (S-slope) receive higher solar radiation (up to 300–800%); it is warmer, drier, more heterogeneous, more unstable and random than the north-facing slopes (NS) (Pavlicek *et al*., 2003). The level of solar radiation has a marked influence on EC biodiversity through its impact on soil, air moisture and temperature. In particular, microclimatic aridity has been implicated as a major evolutionary force on adaptive molecular and organismal evolution in the EC (Nevo, 1991; 1995). Interslope differentiation due to solar radiation intensity gives phenotypically and genotypically different organisms around the Canyon which is considered as a model place to determine the evolution and genetic diversity or similarity among local fungal population (Nevo, 1995; 1997).

Microsatellites or short "simple sequence repeats" (SSRs) are extensively distributed in genome of prokaryotes and eukaryotes and they exhibit extensive level of polymorphism in natural populations. These sequence motifs are made up of variable number of repeats, with each repeat unit of 1–9 bp (Hancock, 1995; Katti *et al*., 2001; Merkel and Gemmell, 2008). However, recent studies have shown that in higher eukaryotic genomes certain tri and hexa-nucleotides are more frequent in coding regions (Borstnik and Pumernik, 2002). Due to insertion or deletion mutations of one or more repeat unit sequence polymorphism take place in these SSRs loci (Tautz and Renz, 1984). It has been evident from several studies that there is strong link between SSR proportion and size of genome. According to Morgante *et al*. (2002) SSR richness is reciprocal to the genome size, where as other suggests a transparent correlation between total genetic makeup of an organism and SSR abundance. Moreover, the richness of SSRs varies between different taxa (Hancock, 1995; Kunzler *et al*., 1995).

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In 1996 a new approach based upon the bioinformatics tools for studying microsatellite evolution opened up with the study whole genome of *Saccharomyces cerevisiae* (Goffeau *et al*., 1996; Perez *et al*., 2001). In the genomic era, microsatellite distribution can easily be detected bioinformatically (Fondon and Garner, 2004) and flanking primers designed.

Limited information is available on SSRs in fungi due to inadequate research conducted so far on these ubiquitous sequences in this kingdom. It has been reported by Haydar *et al.* (2005) in a survey on SSRs of sequenced fungal genomes that their comparative occurrence is high in human genome as compared to fungi and longer simple sequence repeats are also unique in fungi. This overview equally gives a typical foundation of genetic markers that could be helpful for population genetics and to recognize the different species and isolates of organisms and fungi. The SSRs act as codominant Mendelian markers that are more frequent and reproducible than dominant markers and are extensively used to find out the genetic makeup of population (Taylor *et al*., 1999; Brondani *et al*., 2000). In different genomes the frequencies of various microsatellites have been assessed by hybridization or database searches. We aimed to take advantage of the *macrospora* genome sequence and available bioinformatics tools to develop SSRs for *S. fimicola* and to investigate population structure in the EC.

Materials and Methods

Isolation and Strains Detail

Stock cultures of parental strains of Ascomycete fungus *S. fimicola* were provided by Molecular Genetics Research Laboratory, Botany, Punjab University, Lahore, Pakistan. These cultures were originally isolated from the dung and soil samples of seven assigned stations at the Evolution Canyon, Israel: three on each slope (lower, middle and upper), from three different elevations at 60, 90 and 120 m above sea level and one at the bottom of the valley. The wild isolates were obtained at vegetative phase (i.e., not from ascospores), from soil samples and isolated by adopting the serial diluting method of Waxman (1916). By the humid chamber method fungal samples were isolated from horse dung at 25°C: under aseptic conditions, waste was put in sterilized petri plates with sterilised filter-paper discs soaked in autoclaved distilled water, and desired fungus was recognized by its sporulation method (Domasch *et al.*, 1980). All the stock cultures were subcultured under sterile conditions and maintained on Potato Dextrose Agar (PDA) at 18°C for two weeks to rise the F1 generation by single spore culture method (Table 1).

Genomic DNA Extraction

DNA of 50 strains of *S. fimicola* was extracted from mature perithecia by using CTAB method (Pietro *et al*., 1995). The genomic DNAs along with marker DNA were run on 1% agarose gel stained with ethidium bromide solution and visualized using UV illumination and photographed under gel documentation system. The DNAs were quantified by using HyperLadder™ II (Bioline).

SSR Analysis

The online WebSat program (http://wsmartins.net/websat/) was used to detect SSRs from the *S. macrospora* genome and design primers flanking SSR motifs. The nature of the SSR motifs and the flanking primer sequences are listed in Table 2. These primers were used to amplify the homologous regions in eight positive control strains of *S. fimicola* (S1, S2, S3, N5, N6, N7, Sw92.1 and Sw17.2) and then to amplify all fifty strains

Real Time PCR Analyses

PCR amplifications were carried out using the real time PCR (Roche LightCycler® 480 systems). The 10 (µL) PCR mixture comprised of following reagents (genomic DNA; 5X PCR buffer (Bioline); $MgCl_2$; dNTPs; the LightCycler® 480 High Resolution Melting Master solution (Roche); the IMMOLASE ™ DNA polymerase (Bioline); the forward and reverse primers and double distilled-water).

The amplification was programmed as follows: initial DNA denaturing step of 95 ºC for 10 min, followed by 50 cycles of denaturation (95ºC for 5 s), (65ºC for 15 sec) and (72ºC for 1 min), ending with a final elongation step at 72ºC for 5 min. Fluorescence acquisition was obtained after each 72ºC step. Products were heated to 95ºC for 1 min, cooled to 40ºC for 1 min. and raised to 78ºC for 1 sec. As temperature increased gradually from 78ºC to 95ºC, fluorescence data were acquired continuously.

Results

Genomic sequences of *S. macrospora* (k-hell whole genome shotgun sequence assembly, scaffold_98) under the accession number NW_003546144.1 were drawn from NCBI Genbank data analysis tool to detect regions containing short sequence repeats. A total of 16 regions containing (mono, di, tri, tetra, penta and hexa) nucleotide SSRs were detected using online WebSat (SSRs detector software). Sixteen pair of primers were designed on the basis of the *S. macrospora* genome sequence and shown effective to amplify the targeted region of the expected length (Table 2).

To authenticate the significance of these PCR amplicons, few amplicons were sent for direct sequencing. Twelve of 16 homologues region encompassing targeted SSRs were successfully genotyped with varied number of repeats in strains from both opposing slopes. (CAT)7, (TTG)7, (T)14, (GAA)7, (CGT)6, (AAGCAC)6, (GT)5, (GGC)6 (ACA)8, (CTG)7 (CT)6 and (CAGGGG)5.

Table 1: List of P`arental Strains and their F_1 generation used for evaluation of potential SSR markers for S. *fimicola* collected from two contrasting slopes SFS and NFS of EC

S. No.	S2	S3	N6	N5	S_W 92.1
1.	RS_2 34.6	RS_3 04.2	RN_6 17.2	RN_5 06.5	RS_W 80.1
2.	RS_2 69.1	RS_3 42.3	RN_6 10.5	RN_5 10.7	RS_W 76.2
3.	RS_2 22.2	RS_3 06.3	RN_6 30.1	RN_5 15.5	RS_W 66.5
4.	RS_2 11.3	RS_3 14.5	RN_6 54.5	RN_5 27.4	RS_W 60.7
5.	RS_2 46.4	RS_3 27.6	RN_6 56.8	RN_5 42.3	RS_W 90.3
6.	RS_2 40.1	RS_3 09.2	RN_6 61.3	RN_5 19.6	RS_W 92.5
7.	RS_2 56.8	RS_3 15.7	RN_6 70.7	RN_5 07.8	RS_W 43.8
8.	RS_2 86.7	RS_3 21.8	RN_6 80.4	RN_5 20.2	RS_W 50.5
9.	RS_2 71.5	RS_3 31.5	RN_6 33.7	RN_5 04.1	RS_W 87.1
10.	RS_2 11.6	RS_3 36.1	RN_6 46.2	RN_5 05.5	RS_W 89.3

RS, RN, RSw, indicating progeny raised by Rabia from a single spore of original Strains of S_2, S_3, N_5 N_6 S3, S_W 92.1. RS_2 (F_1 generation of S_2), RS_3 (F_1 generation of S_3), RN_5 (F_1 generation of N_5), RN_6 (F_1 generation of N_6) and RS_W (F_1 generation of S_W 92.1). First number indicated octade number, full stop and second number referred position of spore in an ascus. For initial screening only eight parental strains (S1, S2, S3, N5, N6, N7, Sw17.2 and Sw92.1) were used. Sw stands for Sordaria white mutants

Table 2: List of 16 SSR primer pairs for initial screening to evaluate potential short sequence repeat markers in *S. fimicola*

Primer ID	Target SSR motif	Primer sequence 5' to 3'	Efficiency	Top Blast Hit	Estimated product Size bp
SFSSR-1F	(CAT)7	CCCCTTTCTTGTATTGTCTTGG	SSR	*S. macrospora*	349
SFSSR-1R		GCTGCCTATGTCTTCTTGCTTT	fond		
SFSSR-2F	(CT)6	ACGCCACTACGGAAA ACCTATG	SSR	S. macrospora	361
SFSSR-2R		GCTGCCTATGTCTTCTTGCTTT	fond		
SFSSR-3F	(TTG)7	GTTGTTGCTGTTGTTGTTG	SSR	*S. macrospora*	351
SFSSR-3R		CATGTTCAGCCTCGTCAGGTC	fond		
SFSSR-4F	(A)10	GGCGATAATTTTGGACCTGTAT	Failed to work		350
SFSSR-4R		AGGAAAAGCAAGAAGACGATTG			
SFSSR-5F	(T)14	AACTTCGTGTCAACCTTTGCTT	SSR	*S. macrospora*	398
SFSSR-5R		AGGAGATAAGATGGGATGGGAT	fond		
SFSSR-6F	(GAA)7	TCGGGTTTCTATTGGCTTTTAG	SSR	*S. macrospora*	337
SFSSR-6R		CTCGCCTGTGTGTATCGTATCG	fond		
SFSSR-7F	(CGT)6	TGATGGGGTGGTGTA GAGGTA	SSR found	*S. macrospora*	364
SFSSR-7R		ATTACATGGTCAACAAAAGGCG			
SFSSR-8F	(A)21	AAGA GGCTAATTCA GGAACACG	Failed to work		
SFSSR-8R		AAACAGAGAAAAGAGCAATCGG			
SFSSR-9F	(C)13	CTTCTTCTGCTCTTGGACCTGT	Failed to work		357
SFSSR-9R		TTCTCAACGTAACACCAACGAC			
SFSSR-10F	(AAGCAC)6	TCAACGAACAAACTACCTCCCT	SSR	S. macrospora	350
SFSSR-10R		AATATCCAATGTAACCGCCATC	fond		
SFSSR-11F	(GGC)6	CTGCTACGAGGGAACTGG TTAT	SSR	S. macrospora	362
SFSSR-11R		GCAACTCATGCTGCTCTCATAC	fond		
SFSSR-12F	(CAGGGG)5	AAGACCGTATCGAAGCTCAAAG	SSR	S. macrospora	354
SFSSR-12R		ACCCACTACGTCACCCATAATC	fond		
SFSSR-13F	(GGC)6	ACGCTGGT ATTCGGATTT AGG	Failed to work		367
SFSSR-13R		ACTCTGACCTTGACTTTGACCC			
SFSSR-14F	(CTG)7	CACATAGCTG TACGGTCTGG AT	SSR	S. macrospora	370
SFSSR-14R		TAGTCCCAAGCAAACTGCAATA	fond		
SFSSR-15F	(GT)5	TTG ATTGATACACCAGACAGGG	SSR	S. macrospora	360
SFSSR-15R		ACAGAACAAGGGGTACAGGATG	fond		
SFSSR-16F	(ACA)8	CTCA GATAGCATGGACTGCAAC	SSR	S. macrospora	342
SFSSR-16R		CTTGGTTTTGGGCTTGATTTAG	fond		

Our sequence analysis indicated that although the *S. fimicola* amplicons showed high homology to their corresponding regions in *S. macrospora* and subsequent repeats were in agreement with the available genome and enclosed the predictable number of SSRs. DNA variation (presence or absence of targeted repeats) and variation in number of repeats was evident on strains of both sides followed by alignment with reference sequence using ClustalO (multiple sequence alignment) revealed Insertion of A(T) was found at position 52 (Table 3) in all the strains of SFS amplified by SFSSR primer (1F/1R), while this region was not present in N7, Sw92.1, Sw17.2 (Fig. 1) and their respective progeny. Similarly primer SFSSR (3F/3R) amplified (TGT)16 in all strains of SFS (Table 4), while (TGT)13 in all strains of NFS and also amplified (TTG)15 in all the strains (Fig. 2). (T)14 mono repeats were amplified in N7, N5 and N6 (Table 5) strains while T(A) point mutation was observed at position 57 in (S1, S2 and S3) strains with (T13) mono repeats amplified by primer SFSSR (5F/5R).

Table 3: CLUSTAL O(1.2.3) multiple sequence alignment of parental strains of *Sordaria fimicola* with reference sequence of Sordaria macrospora for (CAT)7 SSR loci. Highlighted area indicating the position of primer. Underline indicating position of targeted SSR

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N5      ------TTTCTTGTATTGTCTTGGACATCTTACGCCCATCATCATCATCATCATCATCCACA 56
S1      CCCCTTTCTTGTATTGTCTTGGACATCTTACGCCCATCATCATCATCATCAACATCCACA 60
S2      CCCCTTTCTTGTATTGTCTTGGACATCTTACGCCCATCATCATCATCATCAACATCCACA 60
S3      CCCCTTTCTTGTATTGTCTTGGACATCTTACGCCCATCATCATCATCATCAACATCCACA 60
N6      CCCCTTTCTTGTATTGTCTTGGACATCTTACGCCCATCATCATCATCATCATCATCCACA 60
Ref     CCCCTTTCTTGTATTGTCTTGGACATCTTACGCCCATCATCATCATCATCATCATCCACA 60
                ************************************************************ ***********

N5      CGGTAGGTTTCCAACCGTTTCCGGAATTCACCGACTTGGTTCAGGTATGATTTCCCTAAC 116
S1      CGGTAGGTTTCCAACCGTTTCCGGAATTCACCGACTTGGTTCAGGTATGATTTCCCTAAC 120
S2      CGGTAGGTTTCCAACCGTTTCCGGAATTCACCGACTTGGTTCAGGTATGATTTCCCTAAC 120
S3      CGGTAGGTTTCCAACCGTTTCCGGAATTCACCGACTTGGTTCAGGTATGATTTCCCTAAC 120
N6      CGGTAGGTTTCCAACCGTTTCCGGAATTCACCGACTTGGTTCAGGTATGATTTCCCTAAC 120
Ref     CGGTAGGTTTCCAACCGTTTCCGGAATTCACCGACTTGGTTCAGGTATGATTTCCCTAAC 120
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N5      CTTGCAATTCCTAGAAATGACCACCCCAACCGAGTTCCACTCCTTTAACCAGCTCCCCTA 176
S1      CTTGCAATTCCTAGAAATGACCACCCCAACCGAGTTCCACTCCTTTAACCAGCTCCCCTA 180
S2      CTTGCAATTCCTAGAAATGACCACCCCAACCGAGTTCCACTCCTTTAACCAGCTCCCCTA 180
S3      CTTGCAATTCCTAGAAATGACCACCCCAACCGAGTTCCACTCCTTTAACCAGCTCCCCTA 180
N6      CTTGCAATTCCTAGAAATGACCACCCCAACCGAGTTCCACTCCTTTAACCAGCTCCCCTA 180
Ref     CTTGCAATTCCTAGAAATGACCACCCCAACCGAGTTCCACTCCTTTAACCAGCTCCCCTA 180
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N5      TCAGCTGAGAGACAAGATCTGGAAGCTCGTCCTCCGGCCGCTTGATCGCGCGGGAGCCCA 236
S1      TCAGCTGAGAGACAAGATCTGGAAGCTCGTCCTCCGGCCGCTTGATCGCGCGGGAGCCCA 240
S2      TCAGCTGAGAGACAAGATCTGGAAGCTCGTCCTCCGGCCGCTTGATCGCGCGGGAGCCCA 240
S3      TCAGCTGAGAGACAAGATCTGGAAGCTCGTCCTCCGGCCGCTTGATCGCGCGGGAGCCCA 240
N6      TCAGCTGAGAGACAAGATCTGGAAGCTCGTCCTCCGGCCGCTTGATCGCGCGGGAGCCCA 240
Ref     TCAGCTGAGAGACAAGATCTGGAAGCTCGTCCTCCGGCCGCTTGATCGCGCGGGAGCCCA 240
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N5      CTTCTTCAACGTCGAGGAACGAGAAAAGCAAGAAGACATAGGCAG 280
S1      CTTCTTCAACGTCGAGGAACGAGAAAAGCAAGAAGACATAGGCAG 285
S2      CTTCTTCAACGTCGAGGAACGAGAAAAGCAAGAAGACATAGGCAG 285
S3      CTTCTTCAACGTCGAGGAACGAGAAAAGCAAGAAGACATAGGCAG 285
N6      CTTCTTCAACGTCGAGGAACGAGAAAAGCAAGAAGACATAGGCAG 285
Ref     CTTCTTCAACGTCGAGGAACGAGAAAAGCAAGAAGACATAGGCAG 285
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Discussion

The present study represents the first attempt to employ molecular markers to explore the genetic diversity of *S. fimicola* in natural populations. The level of genetic variation of a species is widely regarded as an important proxy of the species' adaptability. The variation measurement can be dependent on the number and variability of genetic markers (Eckert and Barrett, 1993). There is no geographical barrier between slopes, but EC showed highly divergent climatic and environmental conditions. It is believed that population performance is significantly related with genetic variation and natural selection which increase fitness of organism (Frankham, 2005). The ascomycete's soil fungus *Emericella nidulans* reveal high polymorphism in stress and arid environment when SSR markers applied (Hosid *et al*., 2008). Saleem *et al*. (2001) also reported higher incidences of crossing over and gene conversion were in the strains isolated from SFS than strains from the NFS. We tested within-slope, as well as between-slope variation and the SSR results presented in this paper are consistent with data from other nuclear markers on EC but found fewer variations as compared to other workers. It is tempting to speculate that the ability to adapt to contrasting microclimates in *S. fimicola* might be associated with genetic variability. Alternative means, such as robust epigenetic mechanisms or high phenotypic plasticity, might have a bigger role in environmental adaptation in this species. A highly similar structure of *S. fimicola* populations and their genetic similarity on both slopes the N and S slopes of the EC might be explained by migration exchange, which is expected for this species because of its ascospores dispersal method. We can best explain this genetic similarity of *S. fimicola* by phenotypic plasticity. It permits organisms to fit themselves according to varied surroundings. It helps to cope with all types of ecologically persuaded changes that could conceivably be lasting all through an individual's life expectancy.

Greater variations on the SFS are normally considered as a versatile method to adapt to its higher biological heterogeneity and stress (Satish *et al*., 2001). Actually, polymorphisms or variations was greater on the more varied and drastic SFS in 11 of 14 model organisms investigated, including wild grain, flies, plants and cyanobacteria. This could highlight the significance of stressed environment and its impact on the hereditary framework (Lamb *et al*., 1998).

Table 4: CLUSTAL O(1.2.3) multiple sequence alignment of parental strains of *Sordaria fimicola* with reference sequence of Sordaria macrospora for (GTG)7 SSR loci. Highlighted area indicating the position of primer. Underline indicating position of targeted SSR

Ref	CATGTTGTTGCTGTTGTTGTTGCACATTTGTTGTTGTTGTTGTTGTCAAGAGAGGTT	60
S1	CATGTTGTTGCTGTTGTAGTTGCACATTTGTTGTTGTTGTTGTTGTCAAGAGAGGTT	60
S2	CATGTTGTTGCTGTTGTAGTTGCACATTTGTTGTTGTTGTTGTTGTCAAGAGAGGTT	60
S3	CATGTTGTTGCTGTTGTAGTTGCACATTTGTTGTTGTTGTTGTTGTCAAGAGAGGTT	60
N5	CATGTTGTTGCTGTTGTTGTTGCACATTTGTTGTTGTTGTTGTTGTCAAGAGAGGTT	60
N7	CATGTTGTTGCTGTTGTTGTTGCACATTTGTTGTTGTTGTTGTTGTCAAGAGAGGTT	60
Sw92.1	CATGTTGTTGCTGTTGTAGTTGCACATTTGTTGTTGTTGTTGTTGTCAAGAGAGGTT	60
	**********	
Ref	GCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTGTTTTTGTTGTTGTTGTTGTTGTTGTCAA	120
S1	GCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTTTTGTTGTTGTTGTTGTTGTTGTCAA	120
S2	GCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTTTTGTTGTTGTTGTTGTTGTTGTCAA	120
S3	GCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTTTTGTTGTTGTTGTTGTTGTTGTCAA	120
N5	GCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTTTTGTTGTTGTTGTTGTTGTTGTCAA	120
N7	GCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTTTTGTTGTTGTTGTTGTTGTTGTCAA	120
Sw92.1	GCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTTTTGTTGTTGTTGTTGTTGTTGTCAA	120
	**********	
Ref	GAGAGGTTGCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTGT**TTGTTGTTGTTGTTGTTG**T	180
S1	GAGAGGTTGCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTGT**TTGTTGTTGTTGTTGTTG**T	180
S2	GAGAGGTTGCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTGT**TTGTTGTTGTTGTTGTTGT**	180
S3	GAGAGGTTGCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTGT**TTGTTGTTGTTGTTGTTG**T	180
N5	GAGAGGTTGCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTGT**TTGTTGTTGTTGTTGTTG**T	180
N7	GAGAGGTTGCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTGT**TTGTTGTTGTTGTTGTTG**T	180
Sw92.1	GAGAGGTTGCAGTCCAGTGTTGTTGTTGTTGTTGTTGTTGT**TTGTTGTTGTTGTTGTTGT**	180
	**********	
Ref	TGTTGCAACTGCTGAGGGACCTGACGAGGCTGAACATG 218	
S1	TGTTGCAACTGCTGAGGGACCTGACGAGGCTGAACATG 218	
S2	TGTTGCAACTGCTGAGGGACCTGACGAGGCTGAACATG 218	
S3	TGTTGCAACTGCTGAGGGACCTGACGAGGCTGAACATG 218	
N5	TGTTGCAACTGCTGAGGGACCTGACGAGGCTGAACATG 218	
N7	TGTTGCAACTGCTGAGGGACCTGACGAGGCTGAACATG 218	
Sw92.1	TGTTGCAACTGCTGAGGGACCTGACGAGGCTGAACATG 218	
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Table 5: CLUSTAL O(1.2.3) multiple sequence alignment of parental strains of *Sordaria fimicola* with reference sequence of *S. macrospora* for (T)14 SSR loci. Highlighted area indicating the position of primer. Underline indicating position of targeted SSR

Ref	AACTTCGTGTCAACCTTTGCTTCAAACCTTTCAATAACTGTTACTGGTCACTTTTTTTTT	59
N5	AACTTCGTGTCAACCTTTGCTTCAAACCTTTCAATAACTGTTACTGGTCACTTTTTTTTT	60
N6	AACTTCGTGTCAACCTTTGCTTCAAACCTTTCAATAACTGTTACTGGTCACTTTTTTTTT	60
N7	AACTTCGTGTCAACCTTTGCTTCAAACCTTTCAATAACTGTTACTGGTCACTTTTTTTTT	60
S1	AACTTCGTGTCAACCTTTGCTTCAAACCTTTCAAGAACTGTTACTGGTCACTTTTTATTT	60
S2	AACTTCGTGTCAACCTTTGCTTCAAACCTTTCAAGAACTGTTACTGGTCACTTTTTATTT	60
S3	AACTTCGTGTCAACCTTTGCTTCAAACCTTTCAAGAACTGTTACTGGTCACTTTTTATTT	60
	********** ********** ********** ***	
Ref	TTTTTGGAAGGACACAGATGACATCGATTACCAATAGTGGAAGTAAAACATACTCTGCTG	119
N5	TTTTTGGAAGGACACAGATGACATCGATTACCAATAGTGGAAGTAAAACATACTCTGCTG	120
N6	TTTTTGGAAGGACACAGATGACATCGATTACCAATAGTGGAAGTAAAACATACTCTGCTG	120
N7	TTTTTGGAAGGACACAGATGACATCGATTACCAATAGTGGAAGTAAAACATACTCTGCTG	120
S1	TTTTTGGAAGGACACAGATGACATCGATTACCAATAGTGGAAGTAAAACATACTCTGCTG	120
S2	TTTTTGGAAGGACACAGATGACATCGATTACCAATAGTGGAAGTAAAACATACTCTGCTG	120
S3	TTTTTGGAAGGACACAGATGACATCGATTACCAATAGTGGAAGTAAAACATACTCTGCTG	120
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Differences in SSRs markers have, up till now, depended on the application of electrophoresis techniques that can be both tedious and hard to genotype. Advanced technologies are required to overcome these problems. In this investigation, we evaluated the most recent of the non-gel-based techniques, high-resolution melting (HRM) analysis. HRM is a strategy that screens precisely the diminishing fluorescent interpolating dye during the time spent separation of dss-nucleic acid. The estimation quickly takes after PCR in a one-step, closed tube technique. This study first time successfully utilized this technique for SSRs genotyping for *S. fimicola*. Yang *et al*. (2014) also employed the same technique for genotyping of short sequence repeats in sheep and genotype 4 polymorphic loci in it. HRM has turned out to be a productive and pragmatic approach to recognize variation in nucleotide

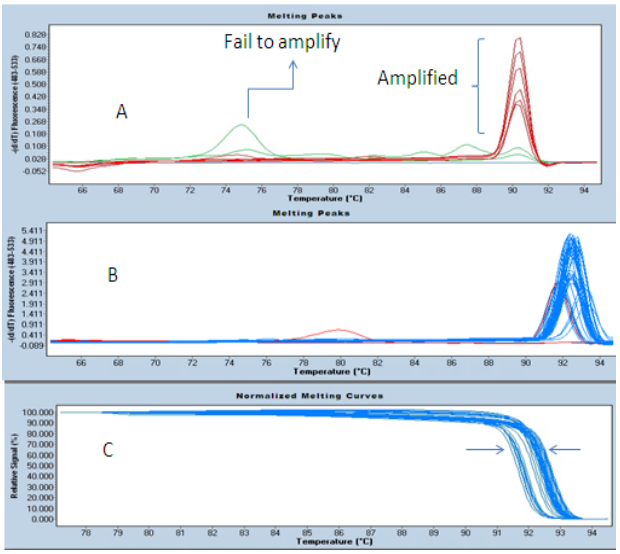

Fig. 1: High resolution melts analysis of *Sordaria fimicola* for genotyping of short sequence repeats (CAT)7 by primer pair SFSSR-1F and SFSSR-1R. (A) Melting Peaks of eight strains of *S. fimicola* for initial screening of (CAT)7 SSR by primer pair SFSSR-1F and SFSSR-1R. (B) Melting Peaks of fifty strains of *S. fimicola* for screening of (CAT)7 SSR by primer pair SFSSR-1F and SFSSR-1R. (C) Normalized melting Curves of fifty strains of *S. fimicola* for screening of (CAT)7 SSR by primer pair SFSSR-1F and SFSSR-1R

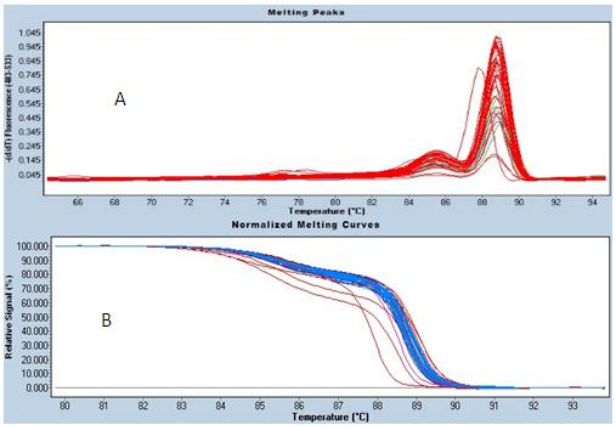

Fig. 2: High resolution melts analysis of *Sordaria fimicola* for genotyping of short sequence repeats (TTG)7 by primer pair SFSSR-3F and SFSSR-3R. (A) Melting peaks of 50 strains of S. fimicola for genotyping of short sequence repeats (TTG)7 by primer pair SFSSR-3F and SFSSR-3R. (B) Normalized melting curves of of 50 strains of S. fimicola for genotyping of short sequence repeats (TTG)7 by primer pair SFSSR-3F and SFSSR-3R

sequences for example, SNPs in all organisms (Derzelle *et al*., 2011) this technique was applied to identify SSRs, in plants Besides, it can likewise be utilized to detect mutation (Li *et al*., 2012).

Conclusion

At this time we employed microsatellite loci to study *S. fimicola* genetic variations and observed polymorphism in motif copy number. SSR diversity may come about because of both environmental and genetic impacts. However, the extensive information concerning genetics and molecular biology of *S. fimicola* is centered on particularly constricted collection of isolate. No substantial modifications were found within the nucleotide sequences between strains of two contrasting environment.

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