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Cross-species transferability of microsatellite markers from *Fusarium oxysporum* for the assessment of genetic diversity in *Fusarium udum*

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Abstract Expressed sequence tags (ESTs) are the source of simple sequence repeats (SSRs) that can be used to develop molecular markers for the study of polymorphism and genetic diversity. In the present investigation, 30 EST simple sequence repeats (SSR) primer sets derived from three *formae speciales* of *Fusarium oxysporum*: *melonis* (*Fom*), *cucumerium* (*Foc*), and *lycopersici* (*Fol*) – were tested for transferability to *Fusarium udum*. The majority of SSR loci contain trinucleotide (63.70%) while fewer contain di- (27.41%), tetra- (5.64%) and penta-nucleotide (3.22%) repeats. The number of alleles at these SSR loci ranged from one to three, with an average of 1.4 alleles per locus. CAG (24.19%) and AC (16.93%) were the most abundant motifs identified. Three markers (*Fom*SSR-8, *Fol*SSR-2 and *Fol*SSR-4) were found highly informative for genetic characterization of *F. udum* and very useful in distinguishing the polymorphism rate of the markers at specific locus; however, polymorphic information content (PIC) was maximum (0.597) in *Foc*SSR-7. In terms of cross species transferability, 70% of the primer sets of *Fom*-SSR and *Fol*-SSR and 30% of the *Foc*-SSR produced an amplicon in *F. udum* isolates. To the best of our knowledge, this is the first set of EST SSR markers developed and assessed for the variability, genetic analysis and evolutionary relationships of the *F. udum* population.

Keywords Co-dominant marker · *Fusarium oxysporum* f.sp. *melonis* · *Fusarium oxysporum* f.sp. *cucumerium* · *Fusarium oxysporum* f.sp. *lycopersici* · Polymorphism

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Introduction

Vascular wilt caused by *Fusarium udum* is an important biotic constraint for sustainable crop production of pigeon pea (*Cajanus cajan*), which has been reported to cause 16-47% crop yield losses (Raju *et al*. 2010). In India alone, the losses due to this disease are estimated to be US $71 million and disease incidence varies from 5.3% to 22.6% (Kannaiyan *et al*. 1984). Use of resistant cultivars is the most effective and economic method to manage the disease. However, a high level of genetic variability among the *F. udum* population and resistant cultivars' selective pressure has led to wide variation in virulence and aggressiveness of the *F. udum* population in the field (Kiprop *et al*. 2005). Pathogenic variability in *F. udum* has been assessed traditionally through virulence tests using a set of host differentials containing different resistance genes. This

is a time-consuming procedure requiring at least 40 days for the analysis, and reactions can be influenced by environmental parameters (Haware & Nene 1982). Therefore, more effective genetic markers are needed to understand genetic variation in *F. udum*.

Molecular markers have become important tools to study and detect genetic variation in a plant pathogen population. Several DNA-based molecular markers such as rDNA-ITS, RFLP, and RAPD have been successfully used for identifying and studying genetic variation and diversity of numerous plant pathogenic fungi (Bogale *et al*. 2006; Mesapogu *et al*. 2012). Simple sequence repeats (SSRs), a more efficient marker system than RFLPs and RAPDs, have been widely developed for genetic analysis of fungi (Barbará *et al*. 2007; Kumar *et al*. 2012; Rouxel *et al*. 2012). SSRs or microsatellites are tandemly repeated DNA sequence units of 1–6 bp. They have abundant and random distribution throughout eukaryotic genomes. Variation in SSR length occurs primarily due to slipped-strand mispairing during replication (Levinson & Gutman 1987) and mutations, which can be detected by PCR with primers designed from the conserved flanking region. Because they are highly polymorphic, multiallelic, co-dominant, PCR-based, and highly reproducible, SSRs provide an ideal molecular marker system for a variety of purposes. A key advantage of EST-SSRs is that they are often more transferable across species as compared with SSRs from non-coding sequences (Pashley *et al*. 2006), thereby facilitating comparative genetic analyses. However, the development of SSR markers from genomic libraries is efficient and relatively inexpensive. With the availability of large numbers of expressed sequence tags (ESTs) and other DNA sequence data through data mining, development of SSRs has become fast, efficient, and cheaper. Recently, EST-SSR markers for three *formae speciales* of *F. oxysporum*: *melonis* (*Fom*), *lycopersici* (*Fol*), and *cucumeris* (*Foc*) – have been developed and utilized for polymorphism studies (Mahfooz *et al*. 2012), but no formal analysis of these microsatellite markers in *F. udum* has been reported.

In view of the above facts, the present study was undertaken to determine cross-transferability of EST-SSRs derived from *formae speciales* of *F. oxysporum* for the assessment of genetic diversity and phylogenetic analysis among *F. udum* isolates.

Materials and methods

Fungal isolates Twenty-eight virulent isolates of Fusaria including 20 of *F. udum*, three of *Fom*, three of *Foc* and two of *Fol* obtained from National Agriculturally Important Microbial Culture Collection (NAIMCC), National Bureau of Agriculturally Important Microorganisms (NBAIM), Mau, Uttar Pradesh, India, were used in the present study. The isolates were selected on the basis of host specificity, geographic origin and pathogenicity (Table 1).

Microsatellite markers and PCR amplification Thirty randomly selected EST-SSR primer sets including ten primer pairs each from *Fom*, *Foc* and *Fol* EST sequence and transcripts (Mahfooz *et al*. 2012) were used for the study of polymorphism and genetic diversity in *F. udum*. Total genomic DNA was extracted using CTAB method (Kumar *et al*. 2013). The PCR was performed in a 10 μl reaction volume containing 1× PCR buffer (10 mM Tris HCl pH 9.0, 1.5 μM $MgCl_2$, 50 mM KCl, 0.01% gelatin), 0.4 mM each of dNTP (Bangalore Genei, Chennai, India), 0.2 U of *Taq* DNA polymerase (Bangalore Genei), 10 pM each of forward and reverse primers and 25 ng of genomic DNA were used as a template. The PCR program was initial denaturation at 95°C for 3 min, and subsequently five touch-down PCR cycles comprising 94°C for 20 s, 60/55°C (depending on the marker as given in Table 3) for 20 s, and 72°C for 30 s. These cycles were followed by 40 cycles of denaturation at 94°C for 20 s with a constant annealing temperature of 56/51°C (depending on marker) for 20 s, and extension at 72°C for 20 s, and a final extension at 72°C for 20 min. PCR amplicons were examined on 3% agarose gel using ethidium bromide staining. 100 bp DNA ladder (MBI Fermentas, Amherst, NY, USA) was used to estimate the allele size.

Diversity and cluster analysis The amplification data generated by SSR markers were analyzed using SIMQUAL route to generate Jaccard's similarity coefficient (Jaccard 1908) using NTSYS-PC, software version 2.1 (Rohlf 1998). These similarity coefficients were used to construct a dendrogram depicting genetic relationships among the isolates by employing the Unweighted Paired Group Method of Arithmetic

Table 1 Details of the isolates of *Fusarium* species used in the study

Code no.	Accession No.	Culture	Biological Origin	Geographical Region	Virulence*
Fu1	NAIMCC-F-02854	*F. udum*	*Cajanus cajan*	Hyderabad, Andhra Pradesh	+++
Fu2	NAIMCC-F-02853	*F. udum*	*C. cajan*	Faridkot, Punjab	+
Fu3	NAIMCC-F-02852	*F. udum*	*C. cajan*	Hissar, Haryana	+
Fu4	NAIMCC-F-02860	*F. udum*	*C. cajan*	Latur, Maharashtra	+++
Fu5	NAIMCC-F-02850	*F. udum*	*C. cajan*	Ranchi, Jharkhand	++
Fu6	NAIMCC-F-02849	*F. udum*	*C. cajan*	Mujaffarpur, Bihar	+++
Fu7	NAIMCC-F-02851	*F. udum*	*C. cajan*	Berhampur, West Bengal	++
Fu8	NAIMCC-F-02844	*F. udum*	*C. cajan*	Aligarh, Uttar Pradesh	+++
Fu9	NAIMCC-F-02847	*F. udum*	*C. cajan*	Jabalpur, Madhya Pradesh	+
Fu10	NAIMCC-F-02842	*F. udum*	*C. cajan*	IIPR Kanpur, Uttar Pradesh	+++
Fu11	NAIMCC-F-02855	*F. udum*	*C. cajan*	Guntur, Andhra Pradesh	+
Fu12	NAIMCC-F-02848	*F. udum*	*C. cajan*	Sagar, Madhya Pradesh	++
Fu13	NAIMCC-F-02845	*F. udum*	*C. cajan*	Bahraich, Uttar Pradesh	+++
Fu14	NAIMCC-F-02843	*F. udum*	*C. cajan*	Varanasi, Uttar Pradesh	+++
Fu15	NAIMCC -F-02861	*F. udum*	*C. cajan*	Badnapur, Maharashtra	+++
Fu16	NAIMCC -F-02857	*F. udum*	*C. cajan*	Bangalore, Karnataka	+
Fu17	NAIMCC -F-02858	*F. udum*	*C. cajan*	Gulberga, Karnataka	++
Fu18	NAIMCC -F-02859	*F. udum*	*C. cajan*	Aloka, Maharashtra	+++
Fu19	NAIMCC -F-02856	*F. udum*	*C. cajan*	Krishnagiri, Tamil Nadu	+
Fu20	NAIMCC -F-02846	*F. udum*	*C. cajan*	Allahabad, Uttar Pradesh	+++
Fom1	NAIMCC -F-00915	*F. oxysporum* f. sp. *meloni*	*Cucumis sativus*	Kotputli, Rajasthan	+++
Fom2	NAIMCC -F-00916	*F. oxysporum* f. sp. *meloni*	*C. sativus*	Tonk, Rajasthan	+++
Fom3	NAIMCC -F-00922	*F. oxysporum* f. sp. *meloni*	*C. sativus*	Bagpat, Uttar Pradesh	+++
Foc1	NAIMCC -F-00861	*F. oxysporum* f. sp. *cucumerium*	*C. sativus*	Alipur, Uttar Pradesh	+++
Foc2	NAIMCC -F-00863	*F. oxysporum* f. sp. *cucumerium*	*C. sativus*	Tonk, Rajasthan	+++
Foc3	NAIMCC -F-00869	*F. oxysporum* f. sp. *cucumerium*	*C. sativus*	Sikar, Rajasthan	++
Fol1	NAIMCC -F-02785	*F. oxysporum* f. sp. *lycopersici*	*Solanum lycopersicum*	Varanasi, Uttar Pradesh	+++
Fol2	NAIMCC -F-02792	*F. oxysporum* f. sp. *lycopersici*	*S. lycopersicum*	Coimbatore, Tamil Nadu	+++

*+ = less virulent, ++ = moderately virulent, +++ = highly virulent

Averages (UPGMA) algorithm and SAHN clustering. The robustness of the dendrogram was evaluated with a bootstrap analysis performed on the binary dataset using WINBOOT software (version 2.0). The allelic diversity or polymorphism information content (PIC) was measured as described by Botstein *et al*. (1980). PIC is defined as the probability that two randomly chosen copies of gene will be different alleles within a population. The PIC value was calculated with the formula as follows:

$$PIC_i = 1 - \sum_{j=1^n} Pij^2$$

where Pij represents the frequency of the j^{th} allele for marker i, and summation extends over n alleles.

Results

Transferability of SSR markers Thirty primer sets were tested on different isolates of *F. udum*, using *Foc*, *Fol* and *Fom* as control isolates (Table 1). Twenty-one (70%) of them successfully produced at least one bright and distinct amplicon in *F. udum* isolates ranging from 180–700 bp, whereas nine SSR markers

showed no amplification. The highest rate of successful amplification (80%) was achieved from *Fom* primer sets. Transferability of *F. oxysporum* EST primers ranged from 60% (in the case of *Foc* primers) to 70% (in the case of *Fol* SSR primers) in *F. udum* (Table 2). The functional SSR markers, their repeat motif and repeat numbers, primer sequences, PCR annealing temperature, and expected fragment length are described in Table 3. The majority of SSR loci contain trinucleotide (63.70%) or dinucleotide (27.41%) repeats, while fewer contain tetranucleotide (5.64%) and pentanucleotide (3.22%) repeats. Among 21 markers, eight (38.09%) were polymorphic and the remaining 13 (61.9%) were monomorphic. A total of 31 alleles were amplified by 21 markers (Table 2). The number of alleles at each polymorphic SSR locus ranged from one to three, with an average of 1.4 alleles per locus. The number of alleles detected by *Fom*, *Foc* and *Fol* primers was 12, 11 and eight, with an average of 1.5, 1.6 and 1.3 alleles per locus, respectively. Out of 31 alleles, only 18 (58.1%) were polymorphic. The highest number of alleles (3) was detected by *Fom4* and *Foc7* markers, whereas 13 markers (*Fom*1, *Fom*2, *Fom*5, *Fom*6, *Fom*9, *Foc*3, *Foc*5, *Foc*6, *Foc*9, *Foc*10, *Fol*1, *Fol*3, and *Fol*10) were able to detect one allele per locus. Three SSR markers (*Fom8*, *Fol*2 and *Fol*8) showed 100% polymorphism and minimum level of polymorphism (50%) was revealed by *Fom*3 and *Fol*5 markers. Four *Fol* (*Fol*2, *Fol*4, *Fol*5 and *Fol*9), three *Fom* (*Fom*3, *Fom*4 and *Fom*8) and one *Foc* (*Foc*7) markers were highly polymorphic, with a PIC value ranging from 0.133 to 0.594. *Fom*8, *Foc*7 and *Fol*4 with PIC values ≥0.4 were identified as the most informative SSR markers (Table 3).

Diversity and cluster analysis The similarity coefficient values between isolates ranged from 0.30 to 0.97 with a mean of 0.64 for all 406 isolates/SSRs combination used in the present investigation. For microsatellite markers developed from *Fom*, the similarity coefficient between isolates ranged from 0.22 to 1, with 33.1% genetic diversity. Similarly, with *Foc*-SSR markers, the similarity coefficients between isolates ranged from 0.25 to 1, with 42.7% genetic diversity. For *Fol* markers, a similarity coefficient value ranged from 0.44 to 1.0 with the average diversity being 34.5% (Table 3). The highest similarity value was observed between *F. udum* isolates, *F. udum* 17–20 (0.97) followed by *F. udum* 12–13 (0.94). The dendrogram (Fig. 1) constructed on the basis of similarity index resulted in two major clusters. The first cluster is composed exclusively of *F. udum* isolates, and is further divided into many sub-clades. The second cluster is further grouped in two distinct sub-clades, where one clade includes the rest of the *F. udum* isolates, and the second clade includes *formae speciales* of *F. oxysporum* isolates taken into this study.

Discussion

Expressed sequence tags (ESTs) are the source of simple sequence repeats (SSRs) that can be used to develop molecular markers for the study of polymorphism and genetic diversity of the *F. udum* population and related species. Generally, the success rate of EST-SSR primers (percentage of SSR primers producing discrete amplification products) ranged from 50% to 100% between species within genera in plants (Peakall *et al.*

Table 2 Comparison between *Fom*, *Foc* and *Fol* markers in order to estimate the level of transferability and polymorphism among *Fusarium udum*

	Fom SSR	*Foc* SSR	*Fol* SSR	Cumulative Results
Number of SSR primers used	10	10	10	30
Marker amplified (Transferability)	8 (80%)	6 (60%)	7 (70%)	21 (70%)
Number of monomorphic markers	5 (62.5%)	5 (83.3%)	3 (42.8%)	13 (61.9%)
Number of polymorphic markers	3 (37.5%)	1 (16.7%)	4 (57.2%)	8 (38.1%)
Average PIC value	0.363	0.617	0.334	0.438
Number of alleles amplified	12	8	11	31
Average similarity coefficient value	0.61	0.46	0.76	0.61

Table 3 Amplification patterns and polymorphic information content (PIC) of *Fusarium oxysporum* EST-SSR primers in *Fusarium udum*

Primer Name	Primer sequence	Motifs	Temp (°C)	No. of alleles	Expected size (bp)	Observed size (bp)	Polymorphism (%)	PIC
*Fom*1	CTCATCGTCATCGCTATTGCT GAAGAATGGGAACTTAAATGCG	(CAA)4	55.2	1	186	200	-	-
*Fom*2	TCATTCTCCATGTCCTCATCAC TCGTTCCGATAGTAATTCGTCA	(AC)15	55.45	1	179	180	-	-
*Fom*3	ATGCGAAAGAAGGTCTGGATTA GAGAAGCCATTATCAACAACGC	(TC)6	54.5	2	393	400-500	50	0.277
*Fom*4	CTTCGGTTGCTCGACTTTCT ATCCATGATCCCCTAAGATCG	(CTT)4	55.6	3	390	400-700	66.7	0.398
*Fom*5	CGTATCACAGCTACAGCCACTC ATCTCAGTCACCCACTCAACCT	(ACA)4	59.2	1	223	250	-	-
*Fom*6	ACACTCCAAGAACTCAGCATCA GACAAAACTCGCTATTCGTTCC	(AC)6	56.4	1	214	200	-	-
*Fom*8	CAACACACGTCACAATTCTTCC CTTTGGCGACGACCTCCT	(TCG)4	56.2	2	377	500- 600	100	0.476
Fom 9	GCACACAATTCTATCCTCCTCC CTGAAAGTGCTGTTGATACGCT	(CCT)4	57.4	1	200	280	-	-
*Foc*3	CGAAACAATGCGTACATCCAT AAGACTCCATACTCCCGAAACA	(CATT)4	55.2	1	216	220	-	-
*Foc*5	CCCAAAGCAACTACAACGCT ATATCCAAGGAAGTGCAAATGG	(CAG)4	54.9	1	308	380	-	-
*Foc*6	CTGTTTTCTCAAAGACCATGTCC TACACCGATCTCATCAACAAGC	(CGT)4	56.7	1	360	400	-	-
*Foc*7	CAAGTCAGCAACCAACACAACT GTCCTCCCATTCTTCTACCACC	(CGG)4	58.25	3	318	180-300	66.7	0.594
*Foc*9	GTTCGGATCATACAGCACATTT TGGGGAATTAGTACGGAAAAGA	(CT)7	55.5	1	142	200	-	-
*Foc*10	GGCAGGTTTCAATTCTTTGAGT ATCGAACAACGATGGGAGAC	(CAACT)4	56.7	1	158	200	-	-
*Fol*1	GGAGGCCGAGGTAATGGATAC CTGAGACTGAATGGCAGTAGGG	(CGG)7	60.0	1	384	400	-	-
*Fol*2	CTCGCATACTACTACCGCACAG GCAGATAAGGGAGATGCAAAAC	(CAG)10	58.3	2	312	200-300	100	0.42
*Fol*3	AGCAACTGGAGAAAGAATACGC TGATTGGGGTTAGTGAAGGTCT	(GAG)8	56.4	1	325	300	-	-
*Fol*4	CCAGTCAATCCAACCCTTACTT AGGCTTATCTGCGTCAGTTTCT	(ACCA)3	56.4	2	348	200-300	100	0.495
*Fol*5	ACCTAACTCTTGGGAGGACGAT CTGCATAGCCTTGGTTGTTGTA	(CAG)7	57.4	2	308	190- 320	50	0.133
*Fol*9	CATTGGGAGATACGAACACTGA ATTGCGGACTTGAGAACAAAG	(GAC)6	57.15	2	305	200-310	66.7	0.375
*Fol*10	AACAACAGCAACAGCAACAGAT CTTCCAGTAGTGCCAGTGTGAG	(CAG)9	56.2	1	180	200	-	-

1998; Varshney *et al*. 2005), and 34% in cross species transfer of SSRs within genera in fungi (Dutech *et al*. 2007). The 70% amplification of EST-SSRs in the *F. udum* population obtained in the present investigation corroborates the findings of Goodwin (2007), who tested 99 primer pairs designed from the *Mycosphaerella graminicola* EST database on the closely related species *Septoria passerinii* and found that 66% of them amplified. Eight of 12 primer pairs tested also amplified on the more distantly related species *Mycosphaerella fijiensis* (Goodwin 2007). Similarly, Dracatos *et al*. (2006) used 55 primer pairs for EST-SSR loci of *Puccinia coronata* f.sp. *lolii* to amplify the DNA from various fungal species (*Puccinia coronata* f.sp. *avenae*, *Puccinia striiformis* f.sp. *tritici*, *Neotyphodium lolii*, *Blumeria graminis*,

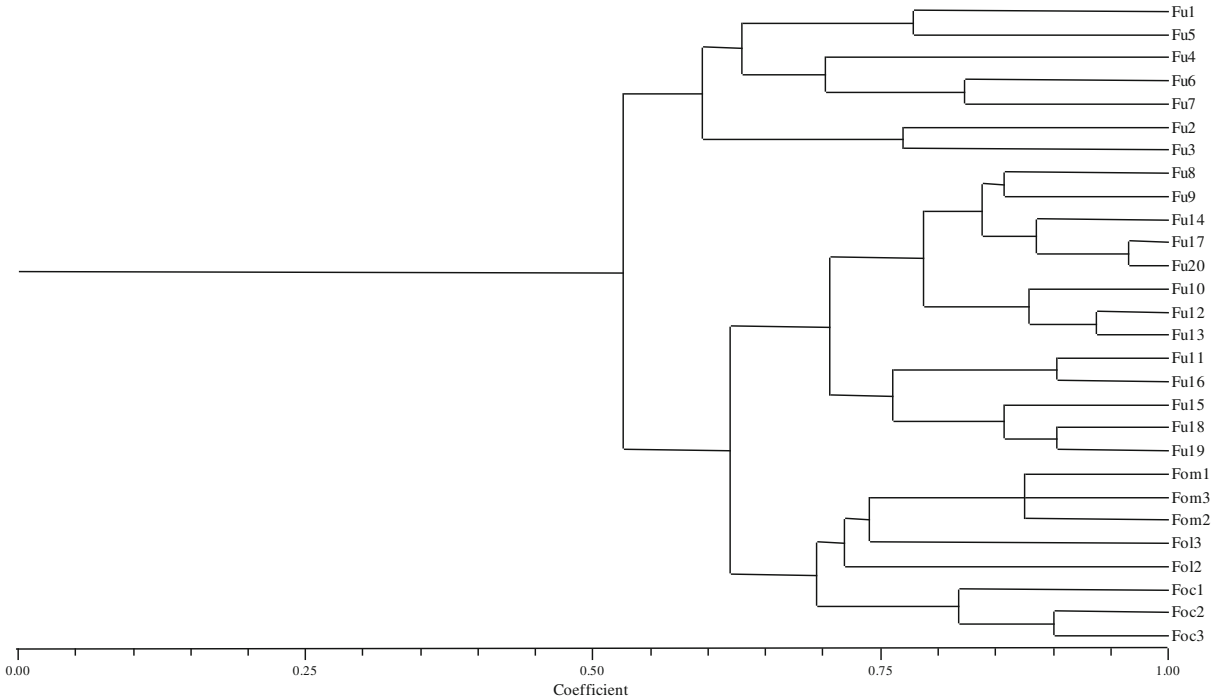

Fig. 1 Dendrogram showing genetic relationship among the *Fusarium udum* and related isolates based on 21 microsatellite markers. Scale indicates Jaccard's coefficient of similarity

Aspergillus nidulans, and *Penicillium marneffei*) and had a success rate of amplification ranging from 22% to 53%. This suggests that SSR primers developed from EST sequences are highly transferable to other related species. The more closely related the organisms, the higher the rate of the transferability due to more closely related species sharing more homology in SSR-containing genes. Another possibility for the high rate of success in the amplification of EST-SSRs may be the result of several factors, such as the sequences from which the primers were derived, the adequate criteria used for primer design and the use of the species of same genus for the design and amplification of the primer set.

Patterns of cross-species SSR amplification in fungi are beginning to emerge, although there are still few studies that systematically explore SSR transferability beyond closely related genera (Dutech *et al*. 2007; Mahfooz *et al*. 2012). In the present study, the EST-SSR markers developed from *formae speciales* of *F. oxysporum*, amplified *F. udum* isolates and exhibited high levels of polymorphism. It is worth mentioning here that a small number of markers (four out of 25) have also been described as transferable from related Uredinales species to *Hemileia vastatrix* (Cristancho & Escobar 2008). The preliminary results obtained in the present study agree with previous reports that describe a smaller fraction of cross species transfer of microsatellites within fungal genera (Baird *et al*. 2010). However, there might be a higher probability of transferability of *Fom*-derived markers than *Foc*- and *Fol*-EST derived markers in *F. udum*, which needs further investigation and statistical validation using a large set of EST-derived SSRs. The results also confirmed wide species transferability of developed EST primers and demonstrated that they may represent a set of well-conserved loci across the species. This may be due to the transfer of lineage-specific genomic regions in *F. oxysporum* (Ma *et al*. 2010).

It has been observed in the study that the distribution of microsatellites in the *F. udum* genome is not random. Tri-nucleotide repeats (CAG and CAA) have been found to be a common feature in EST-derived SSRs. A high frequency of these repeats in coding regions could be due to mutation and selection pressure for specific amino acids. The abundance of tri-nucleotide repeats EST-SSR is likely due to suppression of other kinds of repeats in the coding region,

which reduces the frame-shift mutations in the coding regions (Garnica *et al*. 2006). Additionally, there is a possibility that these tri-nucleotides in the coding region are translated into amino acid repeats (glutamine, proline, arginine, aspartic acid, glutamic acid and serine, etc.), which possibly contribute to the biological function of protein (Kim *et al*. 2008). Di-nucleotide SSRs are often found in the exonic region of *F. udum*, however $(AC)_n$, $(CT)_n$ and $(TC)_n$ repeats are common in all the isolates taken under study. Based on the present status of our knowledge, it is uncertain whether they are merely structural moieties or have some functional significance too.

To analyze the polymorphism pattern in the *F. udum* population, average PIC values were compared and recorded significant distinction in the polymorphism rate of the markers at a specific locus. In this study, 70% of functional SSRs showed polymorphisms in the *F. udum* population, indicating a relatively high level of polymorphism. These markers clustered the *F. udum* population and the other three *formae speciales* of *F. oxysporum* in two distinct major clusters in basal topology. Similar levels of polymorphism have been reported by Mahfooz *et al*. (2012) in *formae speciales* of *F. oxysporum*. Markers with PIC values of ≥0.40, *viz*., *Fom*8, *Foc*7 and *Fol*2, were found to be highly informative for genetic characterization and very useful in distinguishing the polymorphism rate of the markers at a specific locus. The high level of polymorphism associated with SSR is to be expected, because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Varshney *et al*. 2005). The average PIC value was relatively low for SSR markers compared with previous studies in *Fusarium* spp. Bogale *et al*. (2005) developed nine functional SSR markers from *F. oxysporum* having an average PIC of 0.594 and utilized them to discriminate 21 *formae speciales* of *F. oxysproum*. Similarly, Gauthier *et al*. (2007) reported an average PIC value of 0.756 with 15 markers developed from *F. graminearium*. Recently, Mahfooz *et al*. (2012) demonstrated the utility of 30 SSR markers from three *formae speciales* of *F. oxysporum* having average PIC values of 0.53. The lower value of average PIC obtained in the present study may provide an indication that functional SSRs represent the coding region of genome which is generally highly conserved.

In summary, three effective functional SSR markers for the study of polymorphism and genetic diversity in *F. udum* were obtained. These markers have shown a high success rate in PCR amplification and detected a high level of molecular polymorphism in *F. udum* isolates. Additionally, their ease of scoring may facilitate larger studies to compare the evolution of different populations throughout their geographical distribution. In particular, comparison of genetic structure from different *F. udum* populations will be helpful in the understanding of evolutionary dynamics of *F. udum*. Thus, these SSR markers may provide a powerful tool for *Fusarium udum* discrimination, genetic diversity assessment, and genetic relationship studies.

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