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Full Length Research Paper

Development of SCAR marker for specific detection of *Aspergillus flavus*

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***Aspergillus flavus* is the causal agent of corns and peanuts mold known to produce aflatoxin. A quick and reliable PCR-based diagnostic assay has been developed to detect *A. flavus* using a fungus-specific marker derived from genomic DNA. An amplified RAPD product of 600 bp obtained in *A. flavus* isolates using a random primer OPB-11 was cloned in pGEMT easy vector and sequenced. Based on sequences, six primers were designed, out of which a primer pair Asp f1 (CCCGTGAAGTTGCCCAGGT) and Asp r2 (GTCGTTTGGTGAGTGGGAA) amplified a sequence of 490 bp which was specific to *A. flavus*. The specificity of the marker when tested against 31 isolates of *Aspergillus flavus*, 7 isolates of *Aspergillus clavatus,* 4 each isolates of *Aspergillus terreus, Aspergillus oryaze, Aspergillus tamari,* 1 each isolate of *A. parasiticus* and *A. kambarensis* and 4 isolates of 2 different *Cheatomium* species, 5 isolates of 3 different *Trichoderma* species and 5 isolates of 5 different *Fusarium* species showed a specific band of 600 bp only in *A. flavus*. With the optimized PCR parameters, this sequence characterized amplified region (SCAR) marker was sensitive and could detect small quantities of *A. flavus* DNA as low as 10 to 25 ng with high efficiency. This marker could also clearly distinguish *A. flavus* from other fungal plant pathogens, including different *Aspergillus* spp. The utilization of this diagnostic PCR assay in analysis of post harvest samples will be a strong step towards aflatoxin detection in animal feed and export commodity.**

Key words: *Aspergillus flavus*, OPB11, random amplified polymorphic DNA (RAPD), sequence characterized amplified region (SCAR) marker.

INTRODUCTION

Aspergillus flavus is an opportunistic pathogen of agricultural crops, especially oil-containing crops such as maize, peanut and cotton seed. *A. flavus* colonization does not necessarily reduce yield, but causes economic losses by contaminating seed with aflatoxin (Amaike and Keller, 2011). Infection of maize (*Zea mays* L.) by *A. flavus* and the subsequent accumulation of the toxic and highly carcinogenic secondary metabolites, aflatoxins, is a serious agricultural problem, especially in maize grown under dry conditions. Aflatoxin contamination significantly reduces the value of grain both as an animal feed and as an export commodity. It also poses health threats to humans and domestic animals (Chen et al., 2006).

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Aspergillus is a large genus composed of more than 180 accepted anamorphic species, with teleomorphs described in nine different genera. The genus is subdivided into 7 subgenera, which in turn are further divided into sections. As with fungi in general, *Aspergillus* taxonomy is complex and ever evolving. The genus is easily identified by its characteristic conidiophore, but species identification and differentiation is complex, for it is traditionally based on a range of morphological features. Accurate species identification within *A. flavus* complex remains difficult due to overlapping morphological and biochemical characteristics (Rodrigues et al., 2007).

Several species have been described in the past which were assigned to *Aspergillus* section Flavi mainly based on traditional methods (morphological parameters, including colony diameter, colour and texture, size and texture of conidia and conidiophores structure). The *A. flavus* clade includes species characterised with Q-10 (H2) as their main ubiquinone, and conidial colours in shades of

green, and several isolates produce dark sclerotia. *Aspergillus flavus* is the most common species producing aflatoxins, occurring in most kind of foods in tropical countries. This species is very common in maize, peanuts and cotton seed and produces only B-type aflatoxins (Varga et al., 2011).

Phenotypic methods to discriminate *A. flavus* showed only a moderate discriminatory power for distinguishing isolates (Rath, 2001). Genotypic methods that have been used for typing *A. flavus* isolates include RFLP (Moody and Tyler, 1990; James et al., 2000), RAPD (Rath, 2001; Heinemann et al., 2004), microsatellite polymorphism analysis (Guarro et al., 2005) and restriction endonuclease analysis of total cellular DNA has not proven to be a suitable method for discrimination of strains of *A. flavus* (Buffington et al., 1994). James et al. (2000) evaluated a DNA fingerprinting procedure that used a repetitive DNA sequence cloned from *A. flavus* to probe RFLP of genomic DNA. However, RFLP analysis with Southern blotting may be tedious and labour intensive. RAPD analysis is the most frequently applied method, although lack of reproducibility is a well-known limitation of this technique.

RAPD analysis has also been utilized to generate unique PCR products or amplicons in filamentous fungal species or strains of interest to be converted into species- or strain-specific sequence-characterized amplified region (SCAR) markers (Abbasi et al., 1999; Lecomte et al., 2000; Li et al., 1999; Schilling et al., 1996). SCAR markers differ from RAPD markers in that SCAR primers are designed based on known DNA sequences of the organism of study. This allows for the development of sensitive and diagnostic assays to amplify specific fungal DNA in laboratory cultures as well as field samples containing mixed DNA because primers anneal specifically to fungal sequences.

In this study, we utilized RAPD-PCR technique to screen for markers that would differentiate *A. flavus* from other species of the *Aspergillus.* These RAPD markers were converted to SCAR markers for development of a sensitive and reliable diagnostic assay for the selective detection of *A. flavus.*

MATERIALS AND METHODS

Fungal isolates

31 isolates of *Aspergillus flavus*, 7 isolates of *Aspergillus clavatus,* 4 each isolates of *Aspergillus terreus, Aspergillus oryaze, Aspergillus tamari,* 1 each isolate of *A. parasiticus* and *A. kambarensis* and 4 isolates of 2 different *Cheatomium* species, 5 isolates of 3 different *Trichoderma* species and 5 isolates of 5 different *Fusarium* species were obtained from the Indian Type Culture Collection (ITCC), New Delhi, India. Information of host and place for each fungal isolates are listed in Table 1. The fungal pathogens that were culturable on media were grown in 100 ml of potato dextrose broth in a 250 mL flask for five days at 25°C incubation. The mycelia of all the fungal species were harvested and separated by filtration through a Whatman No.1 filter, washed thrice with sterile water, and freeze dried at -40°C until extraction of DNA.

Postharvest samples

Fresh and infected samples of maize kernel and peanut pods were collected from farmers. Fresh samples were surface-disinfected by dipping them in 70% (v/v) ethanol/water for 10 s, then placing them in 0.5% sodium hypochlorite solution for 5 min and rinsed three times in sterile distilled water. For the purpose of inoculation, inoculum from *A. flauvus* was prepared by harvesting the spores from 7-day-old culture grown at 27°C on czapek's agar pored Petri plates. Spore suspension was diluted in sterile water and suspension was adjusted to the concentration of 5×10^4 conidia/ml. A surfactant (Tween 20) was added at a concentration of 100 µl/L to reduce surface tension and prevent spore clumping. Samples were inoculated using a hand atomizer and samples without inoculation were also maintained as control.

DNA extraction from pathogens and post harvest samples

Cultures were maintained on Potato Dextrose agar slants. Fungal genomic DNA for screening RAPD and SCAR markers was isolated from mat grown in potato dextrose broth medium following the method of Culling (1992) with partial modification.

DNA was also extracted from inoculated and uninoculated samples as well as from field samples of infected maize kernel and peanut pods. DNA concentration was determined by the use of a nanodrop (Thermoscientific, USA).

ITS region amplification, RAPD analysis and PCR conditions

All the isolates used in this study were confirmed through the ITS region amplification using ITS 1 and 4 primers (White et al., 1990). A preliminary screening was conducted using five different species of *Aspergillus*, against 10-nucleotide random primers obtained from Operon Technologies. A total of 9 RAPD primers were screened (Table 3), from which one primer which was reproducible, and unique amplicons of 600 bp in *Aspergillus flavuus* was selected. The one primer selected was OPB-11 (5'-GTAGACCCGT -3') and screened against 30 isolates of *A. flavus*, 7 isolates of *A. clavatus,* 3 isolates of *A. oryaze and* 1each isolate of *A. terreus, A. tamari, A. parasiticus* and *A. kambarensis* (Table 1). PCR mixtures (100 µl volume) contained 1XPCR buffer with 15 mM $MgCl_2$ (Genei); 200 µM each of dATP, dCTP, dGTP and dTTP (Genei); 10 pmol RAPD primer; 10 ng fungal DNA; and 2.5U Taq DNA polymerase (Genei) PCR amplification was performed in a Bioer thermal cycler programmed for initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 60 s, annealing at 35°C for 60 s, and extension at 72°C for 2 min; and a final extension step at 72°C for 5 min. Reaction tubes were held at 4°C prior to visualization of PCR products in a 1.2% agarose gel stained with ethidium bromide. Each RAPD assay was done three times to ensure reproducibility.

Cloning and sequencing of strain-specific RAPD amplicons

Amplified DNA fragments were separated on 1.2% agarose gel. The selected diagnostic RAPD marker for *A. flavus* from OPB-11 primer was excised from agarose gels and the DNA fragment was recovered using QIAquick gel extraction kit (Qiagen) following the manufacturers protocol. An aliquot of the recovered DNA fragment was reamplified using the corresponding primer to verify that only a single band was excised. The RAPD markers were cloned using a pGMT easy vector (promega Corporation, USA). Ligations, transformations of *E. coli* XL blue, and plasmid amplifications were performed following standard procedures (Sambrook and Russell, 2001). After cloning, positive colonies were selected and each colony was cultured overnight in Luria-Bertani liquid medium

Table 1. Isolates of different *Aspergillus* spp used to screen primer specificity.

Name of the Isolate	ITCC No	Place	Host
*Aspergillus flavus**	107	ISTI, Kanpur	soil
*Aspergillus flavus**	298	New Delhi	Soil
*Aspergillus flavus**	314	New Delhi	Dead locust
*Aspergillus flavus**	315	New Delhi	Stale bread
*Aspergillus flavus**	325	New Delhi	Dead locust
*Aspergillus flavus**	1419	New Delhi	*Zea mays* rhizhosphere
*Aspergillus flavus**	1466	West Bengal	Soil
*Aspergillus flavus**	1623	New Delhi	Dung
*Aspergillus flavus**	1652	Hyderabad	*Arachis hypogaea*
*Aspergillus flavus**	1653	Hyderabad	*Arachis hypogaea*
*Aspergillus flavus**	1654	Hyderabad	*Arachis hypogaea*
*Aspergillus flavus**	1655	Hyderabad	*Arachis hypogaea*
*Aspergillus flavus**	1670	New Delhi	*Areca catechu*
*Aspergillus flavus**	1717	Hyderabad	*Arachis hypogaea*
*Aspergillus flavus**	1718	Hyderabad	*Arachis hypogaea*
*Aspergillus flavus**	2008	New Delhi	Maize garin
*Aspergillus flavus**	2043	New Delhi	Wheat grain
*Aspergillus flavus**	2392	Nainital	*Glycine* sp. seeds
*Aspergillus flavus**	4008	New Delhi	Paddy
*Aspergillus flavus**	4447	Izatnagar	Soil
*Aspergillus flavus**	4516	Nagpur	Citrus rhizhosphere
*Aspergillus flavus**	4793	CPCRI, Kerala	Insect
*Aspergillus flavus**	5003	CPCRI, Kerala	Opisina insect
*Aspergillus flavus**	5004	CPCRI, Kerala	Stephonitis
*Aspergillus flavus**	5005	CPCRI, Kerala	Opisina insect
*Aspergillus flavus**	5006	CPCRI, Kerala	Proutista
*Aspergillus flavus**	5072	Hyderabad	Soil
*Aspergillus flavus**	5076	Hyderabad	Soil
*Aspergillus flavus**	5192	IARI, New Delhi	Groundnut
*Aspergillus flavus**	5290	Jorhat	Soil
Aspergillus flavus	5477	Agra	Human ear
*Aspergillus clavatus**	454	NRRL, Noida	Soil
*Aspergillus clavatus**	1442	West Bengal	Soil
*Aspergillus clavatus**	6537	New Delhi	Soil
*Aspergillus clavatus**	6538	New Delhi	Soil
*Aspergillus clavatus**	2397	Nainital	*Dolichos* sp. seed
*Aspergillus clavatus**	2597	New Delhi	Pigeon droppings
*Aspergillus clavatus**	2598	Kasaragod, Kerala	Albino soil
*Aspergillus oryzae**	4712	NRRL, Noida	Soil
*Aspergillus oryzae**	4713	NRRL, Noida	Soil
*Aspergillus oryzae**	4714	NRRL, Noida	Soil
Aspergillus oryzae	4885	NRRL, Noida	Soil
*Aspergillus terreus**	297	New Delhi	Soil
Aspergillus terreus	301	Kanpur	Soil
Aspergillus terreus	309	New Delhi	Air
Aspergillus terreus	1641	Mysore	Soil
*Aspergillus tamerii**	1625	New Delhi	Dung
Aspergillus tamerii	2017	New Delhi	Wheat grain
Aspergillus tamerii	2436	Hyderabad	Soil
Aspergillus tamerii	2455	Hyderabad	Soil

Table 1. Contd.

*Aspergillus parasiticus**	456	New Delhi	Soil
*Aspergillus kambarensis**	3843	Nainital	Maize
Trichoderma virens	4177	Lucknow	Mango
Trichoderma viride	2211	New Delhi	Soil
Trichoderma harzianum	2895	Turkey	Soil
Trichoderma virens	4911	Barrackpore	Soil
Trichoderma hamatum	2084	Trichy	Soil
Fusarium oxysporum	6386	Jammu	Soil
Fusarium moniliforme	6385	Hyderabad	Soil
Fusarium solani	6342	New Delhi	Soil
Fusarium graminearum	5320	Karnal	Wheat leaves
Fusarium udum	5241	Varanasi	Soil
Chaetomium globosum	2034	New Delhi	Wheat grain
Chaetomium globosum	2401	Nainital	Soil
Chaetomium indicum	2036	New Delhi	Maize grains
Chaetomium indicum	2402	Hyderabad	Soil

*Isolates used for RAPD analysis of OPB 11 to select the desirable SCAR marker.

Table 2. Primer designed from cloned sequence.

Name of primer	Nucleotide sequence
Asp f1	5'-CCCGTGAAGTTGCCCAGGT–3'
Asp f2	5'- CAGCGAACTTGAACGCGCCA-3'
Asp f3	5'- CTACGTGGATGCGGGCTGTT–3'
Asp r1	5'-TGTCTGACTGCAACCCCGA-3'
Asp r2	5'-GTCGTTTGGTGAGTGGGAA-3'
Asp r3	5'-GGTGCGTAAAGAGCCTGCTG-3'

Table 3. Random amplified polymorphic DNA primers used in the present study.

Primer	Primer sequence
OPB2	TGATCCCTGG
OPB5	TGCGCCCTTC
OPB7	GGTGACGCAG
OPB11	GTAGACCCGT
OPB17	AGGGAACGAG
OPB18	CCACAGCAGT
OPA20	GTTGCGATCC
OPZ19	GTGCGAGCAA
R108	GTATTGCCCT

containing ampicillin at 100 mg/L. The size of cloned fragment was verified by using the corresponding RAPD primer and digested by restriction enzymes in multiple clone site of vector. The recombinant plasmids were extracted using alkaline lysis method (Ausubel et al., 2002), and fragment was sequenced by an automated ABI 3100 Genetic Analyser (Applied Biosystems, USA) by Bangalore Genei (Bangalore, India) using M13 promoter specific primers. The sequences were assembled using Clustal X version 2.0.

SCAR primers and PCR conditions

The nucleotide sequence of the cloned RAPD fragment was used to design pairs of SCAR primers. The different sets of primers were synthesized by the MWG, Europines Banglore, India. PCR amplifications was carried with designed primer pairs and PCR mixtures (50 µl volume) for SCAR assays consisted of 1XPCR buffer with 1.5 mM $MgCl_2$ (Genei); 200 µM each of dATP, dCTP, dGTP and dTTP (Genei); different concentration of Asp f1/Asp r2 primer; varied from 10 to 100 ng fungal DNA; and 2.5U Taq DNA polymerase (Genei). PCR amplification was performed in a Bioer thermal cycler programmed for initial denaturation at 94°C for 60 s ; 35 cycles of denaturation at 94°C for 30, 35, 40, 45 s, annealing at 62°C for 40, 45, 50 s, and extension at 72°C for 40 and 60 s; and a final extension step at 72°C for 10 min. All PCR reactions with designed primers were repeated at least three times and suitable controls were taken. PCR products were visualized in 1.2% agarose gels stained with ethidium bromide.

Specificity and sensitivity test of SCAR marker

Specificity of SCAR primer pair was tested by PCR assays against 31 isolates of *Aspergillus flavus*, 7 isolates of *Aspergillus clavatus,* 4 each isolates of *Aspergillus terreus, Aspergillus oryaze, Aspergillus tamari,* 1 each isolate of *A. parasiticus* and *A. kambarensis* and 4 isolates of 2 different *Cheatomium* species, 5 isolates of 3 different

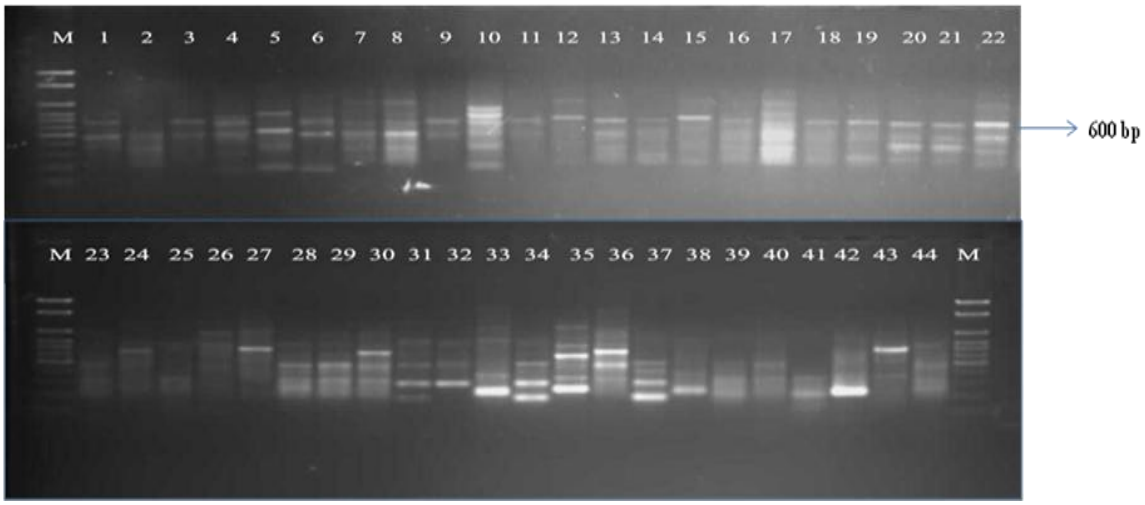

Figure 1. The amplified Polymorphic DNA band with primer OPB11 in isolates of Aspergillus spp Lanes: M 100 bp molecular marker(Bio lab); 1-30 *Aspergillus flavus,* 31-33 *Aspergillus oryzae,* 34 *Aspergillus parasiticus,* 35 *Aspergillus tamarii,* 36 *Aspergillus terreus,* 37 *Aspergillus kambarensis,* 38- 44 *Aspergillus clavatus*.

different *Trichoderma* species and 5 isolates of 5 different *Fusarium* species. The sensitivity of devised SCAR assays was tested by setting up PCR with variable quantities (1 to 100 ng) of *A. flavus* genomic DNA. Sensitivity assays were replicated at least twice.

RESULTS

ITS region amplification, RAPD analyses of *A. flavus* and other *Aspergillus* species

All the isolates used in this study of ITS region were amplified for the confirmation of the presence of the *Aspergillus* species and other genus (Figure 3). Out of the nine RAPD primers screened, PCR products were unique to *A. flavus* among the five *Aspergillus* species used for initial screening. Among the nine primers, OPB-11 was selected for generating well-resolved and reproducible bands that were 600 bp (Figure 1) and thus easily amenable to cloning and sequencing. The selected RAPD primer OPB-11 produced unique amplicons of 600 bp in *A. flavus.*

Development of SCAR markers

To develop a diagnostic assay for *A. flavus*, the only selected RAPD marker were converted to SCAR markers. Six primers were designed (Table 2) based on the sequence of both ends of each of the cloned RAPD marker primer pair sequences, except for those derived from OPB-11:600 bp amplicon. Primer sequence consisted of the 10-nucleotide sequence of the corresponding RAPD primer, followed by a variable num-ber of bases of the amplified sequence. Primer length was determined by compatibility of melting temperatures of the forward and reverse primers.

PCR amplification with these primers in different combinations taking DNA of selected *A. flavus* isolates and other *Aspergillus* spp. as template was performed. The primer pair Asp f1 (5'-CCCGTGAAGTTGCCCAGGT–3') and Asp r2 (5'-GTCGTTTGGTGAGTGGGAA-3') produced the specific band in all tested isolates of *A. flavus* (Figure 2), indicating that this SCAR marker was specific to *A. flavus*. The optimal PCR conditions standardized were as follows: one cycle of denaturation at 94°C for 1 min; followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 62°C for 45 s, and extension at 72°C for 1 min; and a final extension at 72°C for 10 min.

Specificity and sensitivity test of SCAR marker

To test the specificity and efficiency of the SCAR marker, we performed PCR reactions with identified primers using template DNA of all against 31 isolates of *Aspergillus flavus*, 7 isolates of *Aspergillus clavatus,* 4 each isolates of *Aspergillus terreus, Aspergillus oryaze, Aspergillus tamari,* 1 each isolate of *A. parasiticus* and *A. kambarensis* and 4 isolates of 2 different *Cheatomium* species, 5 isolates of 3 different *Trichoderma* species and 5 isolates of 5 different *Fusarium* species (Figure 4). The primer pair displayed high specificity and distinguished clearly *A. flavus* from other *Aspergillus* species and other fungal isolates (Figure 2) by producing an amplicon of 490 bp. This confirmed the species specificity of the developed marker. The sensitivity of the marker using a dilution series of total genomic DNA extracted from *A. flavus* revealed that as little as 10 to 25 ng of template is sufficient for PCR diagnostics.

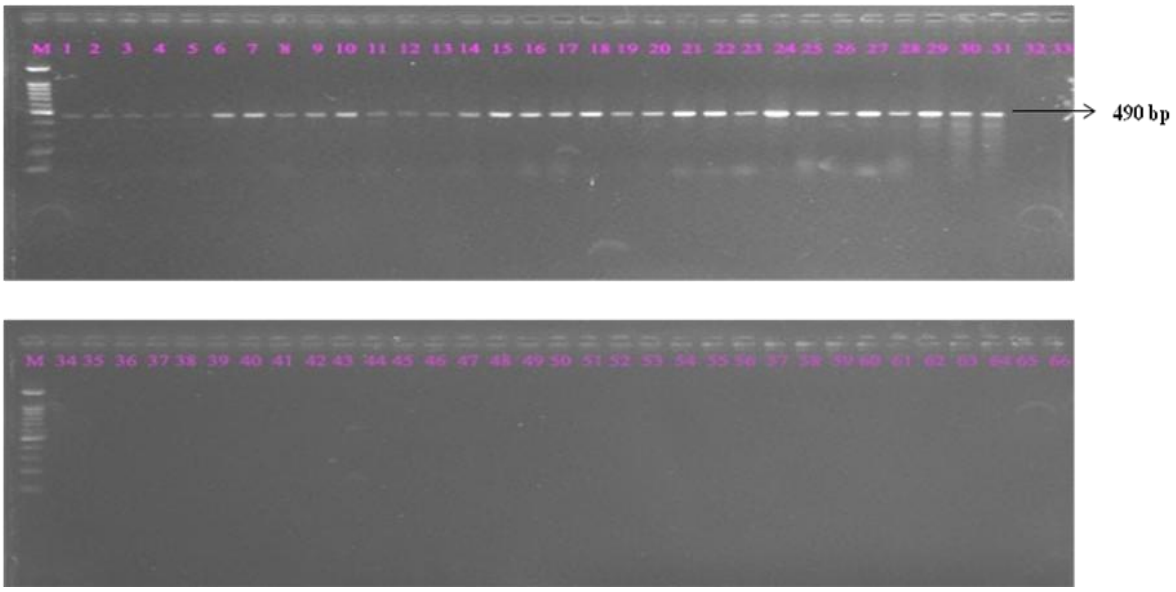

Figure 2. Specificity of primer set Asp f1/Asp r2 to *Aspergillus flavus* using DNA templates of different *Aspergillus* spp and other genera Lanes: M 100 bp molecular marker (Bio lab) 1-31 *Aspergillus flavus* isolates, 32-38 *Aspergillus clavatus* isolates, 39-42 *Aspergillus terreus* isolates,43-46 *Aspergillus tamarii* isolates, 47-50 *Aspergillus oryzae* isolates 51 *Aspergillus parasiticus* isolate, 52 *Aspergillus kambarensis,* 53 -66 *Trichoderma, Fusarium, Cheatomium* isolates.

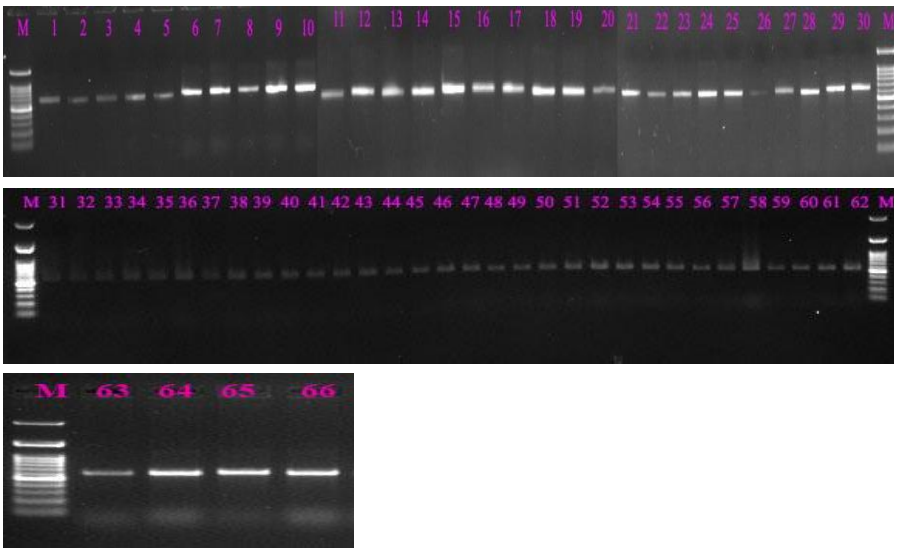

Figure 3. ***Aspergillus*** **spp confirmation through ITS region amplification** using DNA templates of different *Aspergillus* spp and other genera Lanes: M 100 bp molecular marker (Bio lab) 1-31 *Aspergillus flavus* isolates, 32-38 *Aspergillus clavatus* isolates, 39-42 *Aspergillus terreus* isolates,43-46 *Aspergillus tamarii* isolates, 47-50 *Aspergillus oryzae* isolates 51 *Aspergillus parasiticus* isolate, 52 *Aspergillus kambarensis*, 53 -66 *Trichoderma*, *Fusarium*, *Cheatomium* isolates.

PCR detection of *A. flavus* from infected kernel samples of maize and peanuts

The universality of the marker was tested to detect the presence of the pathogen in maize kernel and peanut pod samples. The predicted amplification product of 600 bp was obtained in inoculated samples. Uninoculated samples did not show any amplification. A clear desired band was amplified with DNA from infected maize kernel and peanut pod samples which were collected from

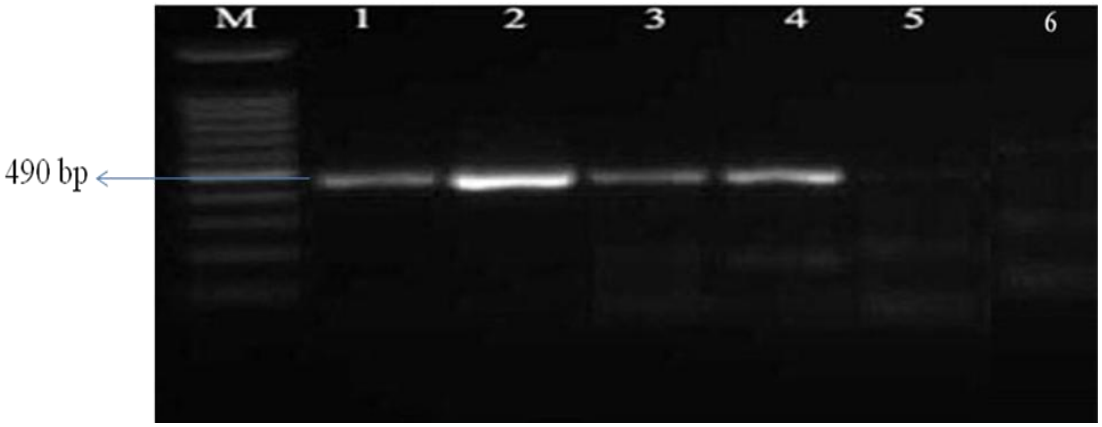

Figure 4. Specificity of primer set Asp f1/Asp r2 to *A. flavus* using DNA templates of infected kernel samples of maize and peanuts collected from retailers and also from farmers. Lanes: M, 100 bp molecular marker (Bio lab); 1, *A. flavus* inoculated samples; 2 to 4, *A. flavus* isolates from infected kernel samples of maize and peanut pod; 5, uninoculated samples; 6, sterile water.

farmers. No PCR amplification negative control (sterile water) was observed (Figure 3).

DISCUSSION

The RAPD technique has been questioned due to its lack of total reproducibility (Büscher et al., 1993; Lamboy, 1994) which is an important pitfall both to identification in routine procedures and to data exchange among laboratories. In the present study, our objective was to clone specific RAPD marker and to transform them into single SCAR marker. The reliability of SCAR markers linked to a unique locus has been reported in some important crops (Naqvi and Chattoo, 1996; Barret et al., 1998) as well as in grapevine (Lahogue et al., 1998). Phenotypic and genetic characterization is important for the efficient identification of *A. flavus.*

In recent years, molecular tools such as RFLP, RAPD, AFLP, MLEE, ribosomal RNA sequences and protein-coding gene sequences have been applied to taxonomic questions in the genus. Multilocus DNA sequence studies of some anamorphic species have shown that the patterns of polymorphisms in different genes are consistent with recombination in these asexual species (Geiser et al., 1998). Single locus DNA sequence studies have been conducted in *Aspergillus* using different loci, and there are extensive databases available for nuclear ribosomal RNA genes (large subunit, internal transcribed spacers) and β-tubulin (Geiser et al., 2007). Any of these gene regions alone may serve as an effective tool for identifying well-defined species, but a limitation of the single locus approach is that not all species can be identified from DNA polymorphisms therein (Geiser et al., 2007).

Phylogenetic studies across the genus have utilised one or a few markers, and unsurprisingly, do not resolve backbone relationships among sections and subgenera (Berbee et al., 1995; Geiser et al., 1996; Ogawa and Sugiyama, 2000; Peterson, 2000).

In order to resolve the central question of species boundaries especially in *Aspergillus flavus,* the RAPD technique has been found to be more useful for detecting genomic polymorphism, as these generate neutral markers that may reflect the whole genotype of an individual.

This study demonstrated that SCAR primers developed from RAPD markers can be used to unambiguously identify *A. flavus*. Also, these SCAR primers proved useful in discriminating *A. flavus* from other *Aspergillus* species.

In previous studies, 20 isolates of *A. flavus* were characterized on the basis of morphology and molecular variability (Alemayehu and Prameela, 2007). However, these criteria cannot be used as suitable markers to verify the identity of *A. flavus* within the genus. A SCAR marker from an anonymous unique region of genomic DNA of *A. flavus* was identified after screening from different species of *Aspergillus* using nine RAPD primers. Primer OPB-11 produced monomorphic band of 600 bp only in *A. flavus,* which was converted into a 490 bp SCAR marker. Primer pair Asp f1 and Asp r2 amplified distinct band of 490 bp with genomic DNA of *A. flavus* and was absent in other *Aspergillus* species.

The other important quality of the marker is its sensitivity, rapidity and reliability. The primers were sensitive enough to accurately amplify as little as 10 to 25 ng of DNA in the PCR assay. *A. flavus* and other *Aspergillus* species capable of aflatoxin production, have considerable diversity. Such diversity makes it more difficult to assign firm taxonomic identity to isolates from such populations (Perrone et al., 2007). In our results, *Asperegillus flavus* isolates were amplified from different hosts like maize grain, wheat grain, paddy fields, citrus rhizhosphere, opsinia insect, groundnut, peanut and human ear by developed Aspf1 and Aspr2 primers.

The PCR based assay developed in this study used for the detection of *A. flavus* from infected samples of maize kernel and peanut pod were collected from retailers and farmers. Results can be obtained within 24 h, when compared with up to several days for conventional methods. The specific assays reported in this work for identification of *A. flavus* provides a simple and efficient tool for early, rapid, sensitive and accurate detection from different hosts.

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