Genetic Diversity of Toxigenic *Fusarium Verticillioides* associated with Maize Grains in India

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Research Article

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Abstract

Genetic diversity of *Fusarium* species associated with maize grains were analyzed from ten states of India. *Fusarium verticillioides* (88.52%), *F. coffeatum* (6.55%), *F. foetens* (3.27%) and *F. euwallaceae* (1.63%) were recovered from the maize samples. A phylogenetic analysis was performed based on the translation elongation factor 1 alpha (TEF1- α) gene sequence formed five evolutionary lineages (EL) that showed both inter- and intra-species variability. A *In-vitro* pathogenicity study confirms that among the 61 strains, 51% exhibited high to moderate virulence to the maize, and produced one or more mycotoxins. The genetic diversity of *F. verticillioides* were evaluated using Inter simple sequence repeats (ISSR) fingerprints. The ISSR fingerprints (AG)8C and (AG)8G shown 252 and 368 microsatellites sites, respectively, in the genome of *F. verticillioides*. The (AG)8C fingerprints differentiated the pathogenic and non-pathogenic clustering of *F. verticillioides*. However, Simpson's (SID), and Shannon's (H) index showed a wide range of diversity within the *F. verticillioides*. The pleomorphic nature of these strains role in host pathogen interactions and adoptive potential to develop disease to crop plants. Toxigenic potential of *F. verticillioides* was evaluated for the presence of FUM1 and FUM13 genes involved in fumonisins B1 (FB1) biosynthesis, and further confirmed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) showed 91% strains were FB1 producers. The current finding demonstrated that most *F. verticillioides* strains showed a wide range of genetic diversity with varied toxigenic and pathogenic potentials. Thus, there is an urgent need to develop an effective resistant breeding program in maize, and further monitoring is necessary for the post-harvest losses of grains.

Introduction

Maize (*Zea mays* L.) is a widely distributed cereal crop after wheat and rice [1, 2]. It is used for various purposes such as livestock feed (45%), human food (11%), and industries (44%) [3]. It has a high nutritional value, starch (60-68%), proteins (7-15%), fat (5-6%), vitamins (Pantothenic acid, folate, vitamin B6, and niacin), and minerals (manganese, phosphorus, magnesium, zinc, and copper). It also has antioxidant activity that protects the cell's DNA from damage and improves digestion [4]. Food and agriculture organization (FAO) predicted that by 2030 the overall demand for maize will increase to around 300 million tons [5]. *Fusarium* is a ubiquitous plant pathogenic fungus that endanger plant growth and consistently adulterate economically important agriculture produce [6, 7]. *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, *F. sporotrichioides*, *F. equiseti*, *F. verticillioides*, and *F. proliferatum* are the predominant species that infect maize, rice, wheat etc., and cause vascular wilt, head blight, root rot, stem canker and plant death [8-10]. *F. verticillioides* is a dominant species, thus affecting the yield, nutritional value, and quality of the grains [11, 12]. *Fusarium* species contaminate stored grains, fruits, cereals, nuts and produce numerous mycotoxins [13-15]. Fumonisins (FUM), Zearalenone (ZEA), Trichothecenes (TRI) produced by *Fusarium* species, known to cause acute to chronic toxicity in humans and animals [16, 17]. The exposure of humans and animals to mycotoxin defiled food could result in teratogenicity, estrogenic effects, carcinogenesis, immune suppression, and neurotoxicity [18].

FB1 is an inhibitor of ceramidase synthase and an important enzyme in sphingolipid biosynthesis. Upon consumption of FB1 contaminated food leads to detrimental effect to the humans and animals. It is hepatotoxic, nephrotoxic, and carcinogenic and causes esophageal cancer [19], neural tube defects (NTD), and congenital cerebral defects [20]. In addition, the intake of toxins infected food grains by humans and animals could result in low nutrient absorption, retarded growth in infants, malnutrition, immunosuppression, and also affects the gut microbiota.

Fusarium is a diverse fungal genus with over 300 species [21]. The species identification is a challenging task for mycologists and food microbiologists due to closely related species and more than 20 species complexes. The barcoding of the internal transcribed spacer (ITS), translational elongation factor 1 α (TEF-1 α), RNA polymerase II subunit (RPB2), and β -tubulin (Bt2) could assist in classifying them within the species complex [22, 23]. TEF1- α is one of the most preferred molecular marker for the sub sections of *Fusarium* species [23]. Furthermore, microsatellite inter simple sequence repeats (ISSR) is an important marker to study the genetic diversity for identifying the highly polymorphic multilocus markers [24]. Microsatellite fingerprint differentiates closely related species complexity, genetic diversity, genome mapping, and evolutionary lineages [25, 11].

lcial (SID) index alpha (TeThe food and feed matrices contaminated with multi mycotoxins, which can be identified using multiplex PCR assays for simultaneous detection in a single analysis[26]. The FUM1 and FUM13 genes involved in the FB1 biosynthesis were located within a 42.5 kb region in *F. verticillioides* genome [27, 28]. The European Commission has set tolerable limits for FUM is 2000-4000 $\mu\text{g}/\text{kg}$ in raw cereals and grains [29]. However, there is a lack of well-regulated efforts for resolving such issues in India [30, 31]. Hence, the present study aimed to assess the genetic diversity of *F. verticillioides* using ISSR fingerprints. Furthermore, FB1 producing *F. verticillioides* were evaluated by PCR and liquid chromatography-mass spectrometry (LC-MS/MS) to ensure the grain quality for the safety of the consumers.

Materials And Methods

Primer designing

Primers were designed for the molecular detection of mycotoxins by targeting their biosynthetic pathway genes such as FUM1 and FUM13 involved in biosynthesis of FB1 using SnapGeneViewer and analyzed with the help of PRIMER-BLAST and BLASTN tools to eliminate, if any non-specific targets in the PCR assay.

Sampling, isolation and identification of *Fusarium* species

A total of 140 stored maize grain samples were collected from the poultry industries of ten states, Maharashtra (MH), Rajasthan (RJ), Madhya Pradesh (MP), Andhra Pradesh (AP), Uttar Pradesh (UP), Bihar (BH), Tamilnadu (TN), Karnataka (KA), Jharkhand (JK), and Uttarakhand (UK), India. The collected

maize samples were analyzed for contamination of *Fusarium* species on modified Spezieller Nahrstoffarmer agar (SNA) medium [32]. The traditional identification of species of *Fusarium* was analyzed based on standard manuals and protocols [33].

Molecular identification of *Fusarium* species

Genomic DNA/RNA isolation

The genomic DNA/RNA was extracted from *Fusarium* species after they achieved optimum growth at 72 h. About 100 mg of mycelia were ground to powder in liquid nitrogen using mortar and pestle. According to the manufacturer's instructions, the genomic DNA/RNA was extracted using DNeasy/RNeasy Plant Mini Kit (Qiagen, Germany). The concentration and purity of extracted DNA/RNA were determined using a Nanodrop Spectrophotometer (Thermo Scientific, USA) and stored at -20°C. For reverse transcriptase PCR, total RNA was transcribed using a high-capacity reverse transcription kit (Thermo Fisher Scientific, USA) and an oligo-dT primer. The first-strand cDNA product was used in the PCR with the TEF1- α gene [34]. Further, the species specificity was confirmed with PCR assay using species-specific primer set Vert 1, and Vert 2 primer set specific to *F. verticillioides* (Supplementary Table 1). The PCR was performed in Applied Biosystem veriti (Thermo Fisher Scientific, USA) with a reaction volume of 20 μ L. The amplification mixture consisted of 1 μ L of each forward and reversed primers (10 μ mol L⁻¹), 1 μ L template, 10 μ LTaq master mix (Jumpstart, Sigma Aldrich), and 8 μ L MilliQ water. The PCR program was set as an initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 95°C for 30 s, 52°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 minutes. After successful amplification, the PCR product was purified and sequencing was performed as reported previously [35].

Molecular detection of FB1 gene by PCR assay

The molecular detection of FB1 was carried out by targeting biosynthetic pathway genes such as FUM1 and FUM13 respective to FB1 production. The PCR was carried out in a 20 μ L reaction mixture containing 50 ng template, 10 μ LTaq master mix, 8 μ L MilliQ water, 1 μ L primers (2 μ mol L⁻¹) for each gene. The PCR cycling was set as an initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94°C for 30 sec, annealing at 52°C for 30 sec and extension at 72°C for 1 min with a final extension of 72°C for 10 min. The PCR products were loaded onto 10g L⁻¹ agarose gel electrophoresis and visualized under the gel documentation system.

Pathogenicity of *Fusarium* species

The effect of *Fusarium* species on the seed germination, growth, and development of maize was determined using Water-agar (WA) method [36]. Maize seeds were surface sterilized with 0.01% HgCl₂

and soaked in 1×10^6 spores/mL for 12 h on an orbital shaker; the seeds were soaked in sterile MilliQ water without spore suspension was used as control. Following incubation, seeds were transferred into plant tissue culture bottles (43 mm) containing water agar (2%) under aseptic conditions and incubated for 15 d at 25 °C in 16 h light and 8 h dark conditions. At the end of the incubation period, root and shoot inhibition was measured. Furthermore, seed germination was evaluated by using 7days grown culture filtrate (CF) of *Fusarium* species [37]. Further, FB1 extraction of all the strains of *Fusarium* was carried out using 50 ml CF of each strain and extracted with equal volume of ACN-MeOH–citrate buffer (12.5/12.5/125, v/v/v) under shaking condition at room temperature for 1 h. The obtained extract was evaporated completely using rotary evaporator (Aditya Scientific, India) and eluted into 200 µl MS grade water. The extract was passed through Sep-Pak column (Waters, Ireland), eluted in 50 µl of MS grade water containing 0.1% formic acid and analyzed for the FB1 detection using LC-MS/MS.

Detection of FB1 by LC-MS

An acquity UPLC system (Waters, USA) was used to perform reverse-phase chromatographic partition of the FB1. Peptide BEH C18 column was used for separation with dimensions 2.1 mm x 150 mm, 1.7 µm particle size, and 4 µL injection volume. The column temperature was set at 40°C, and 8° C was the temperature of the sample manager. The separation was carried out with formic acid (0.1%) and water (A); and formic acid (0.1%) and acetonitrile (B) as a mobile phase. An initial gradient used was 90% A and 10% B for 3 min, and then solvent B was increased linearly to 90% within 10 min and was kept constant for 2 min. Further, solvent B was decreased linearly to 10% in 3 min. To avoid carryover in the next acquisition, the column was washed between two acquisitions. The flow rate was 0.5 ml min^{-1} . Eluents were acquired in Mass Spectrometer (TSQ Quantum Access Max mass spectrometer-Thermo Scientific) in positive electrospray ionization (ESI) mode. Each sample was acquired in triplicates. In the ESI+ mode, the MS spray voltage was 4.2 kV. The capillary temperature was 300°C and probe heating temperature 320°C with the sheath gas at 45 arbitrary units and the aux gas was 12 arbitrary units.

Genetic diversity of *F. verticillioides* using Inter Simple Sequence Repeat (ISSR)

The high frequency occurring *F. verticillioides* isolates were further processed to study the genetic diversity using ISSR markers. The primers were selected based on the polymorphic and reproducible banding patterns to characterize all the *F. verticillioides* isolates; the 20 different ISSR primers containing di or tri-nucleotide repeats were used in the present study (supplementary table 1). The PCR was carried out in a reaction volume of 20 µL. The amplification mixture consisted of 1 µL of forward and reverse primer each (10 µmol L^{-1}), 10 µL Taq master mix (Jumpstart, Sigma Aldrich), 8 µL of MilliQ water, and 40 ng DNA. Sterile MilliQ water was used as a negative control for each experiment. The PCR program was set as an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 sec, 44°C for 45 sec, and 72°C for 2 min with a final extension at 72°C for 7 min. The PCR amplified products were loaded

onto 20g L⁻¹ agarose gel electrophoresis in 1x TAE buffer and visualized under a UV trans-illuminator gel documentation system. The ISSR fingerprint data was analyzed based on the presence or absence of a particular allele indicated as 1 and 0, respectively. However, the pairwise distance among the strains was calculated using Jaccard's coefficient from the binary matrix. The distance matrices were used to cluster the strains by Unweighted Pair Group Method with arithmetic means (UPGMA). Furthermore, to identify the better threshold microsatellite partitions, the Adjusted Rand value was calculated for each UPGMA/Dice dendrograms to compare each possible combination of microsatellite regions.

Data analysis

Annotation of sequenced TEF1- α gene was performed using BlastN with default parameters against the NR database. The blast hit results were filtered based on Maximum Query Coverage with maximum identity and lowest e-value. Preference was given to *F. verticillioides* TEF1- α in cases where there was an identical hit from other strains. Phylogenetic analysis was performed using ClustalW of the TEF1- α gene sequences from the isolates. Results of Clustal W were used as input to construct various trees to identify non-*F. verticillioides* gene clusters. The outliers were removed from the analysis, and distance-based trees were constructed. Interactive Tree of Life online tool was used to construct and visualize trees with Newick format as input for analysis. For ISSR analysis, the presence or absence of amplicon observed at a particular locus was scored as 1 and 0 correspondingly, and pairwise distance among the strains was calculated. Amplicon data was grouped into a 500bp window and provided input to unsupervised hierarchical clustering using the UPGMA method. The resultant file was used to identify Clades based on the distance score. Clustering and visualization were done using MegaX software. The tree file was used as input to the ITOL (Interactive Tree Of Life) online tool and the metadata (Geographical and Virulence) to perform circular tree visualization of the clades.

Results

Isolation and molecular identification of *Fusarium* species

A total of 61 different *Fusarium* isolates were recovered from 140 maize grain samples. The isolated colonies were morphologically identified based on the colony characteristics accompanied by microscopic examination of microconidia and macroconidia. Further, molecular identification of *Fusarium* species was performed using TEF1- α gene sequences, which resulted a single and specific amplicon at 300 bp (Supplementary data). The TEF1- α gene sequences were subjected to BLAST analysis at NCBI database and analyzed for the species-level identification. The molecular evolutionary lineages and phylogenetic tree was constructed using MEGA software version X. Amongst, *F. verticillioides* (88.52%) is the most dominating species, followed by *F. coffeatum* (6.5%), *F. foetens* (3.27%), and *F. euwallaceae* (1.63%). *Fusarium* strains were formed five major clades based on phylogenetic analysis. Clade I, IV and V consist of 15, 16, and 17 strains of *F. verticillioides*. Clade III consists of 4 strains of *F. coffeatum*, and clade II consists of 9 different species strains (Figure 1).

Surprisingly, *F. coffeatum*, *F. foetens*, and *F. euwallaceae* were in between the clade II and III suggest the sequence similarity and closeness of these species, or diversity in the species complexes. The TEF1- α gene sequences showed more than 90% sequence similarity and perfectly aligned into the clades and subclades. Present study revealed that *Fusarium* species have a wide range of diversity within the same species collected from the single host. Furthermore, *F. verticillioides* strains were evaluated using species specific primers showed a single amplicon of 400 bp and conformed the species specificity (Figure 2).

Molecular detection and FB1 production

The early detection of mycotoxigenic fungi in food and feed is of paramount importance. All the obtained *F. verticillioides* strains were processed for detection of FB1 biosynthesis. FUM1 and FUM13 gene specific amplification was observed at 927, and 852 bp (Supplementary data). The most of the isolates shown the presence of both FUM1 and FUM13 genes involved in FB1 biosynthesis. Furthermore, all the strains were analyzed and conformed for the FB1 producers using HPLC/LC-MS/MS. Surprisingly, 90% of the strains were able to produce FB1 confirmed by both molecular and analytical methods (Supplementary data).

Pathogenicity assay of *Fusarium* species

Evaluation of pathogenicity of *Fusarium* isolates were carried out using the WA method. The obtained results indicated a negative effect on seed germination, reduction in the root and shoot growth. For each isolate, mean disease severity (MDS) was measured as low (MSD: < 25%), moderate (MSD: 25-50%) and high (MSD: > 50%). *F. verticillioides*, *F. coffeatum*, *F. foetens*, and *F. euwallaceae* showed diversification in their pathogenicity (Table 1). The results showed that nine isolates as high disease severity, affecting maize growth; twenty-two isolates as moderate disease severity, and twenty six showed low disease severity with 11 and 24% inhibition of both root and shoot growth, respectively. Interestingly, 4 isolates of *Fusarium* species did not reveal any disease severity either on seed germination or shoot and root growth inhibition compared to untreated control. The highly pathogenic nine isolates were collected from MH, AP, RJ, JK, and MP, and four non-pathogenic isolates were from MP, RJ, and the UK. The rest of the isolates obtained from various states exhibited low to moderate pathogenicity. However, more detailed studies also needed for the confirmation of pathogenicity with other maize genotypes.

ISSR fingerprint

The genetic diversity of *F. verticillioides* was determined using microsatellite ISSR fingerprints. The polymorphic nature of all *F. verticillioides* strains were representative of each of the ten states studied in India. Among, the twenty ISSR primers used for this study, two primers (AG)8C and (AG)8G showed 99-100% reproducibility of banding patterns for *F. verticillioides*. The data with other primers were nonreproducible and less polymorphic, were omitted from the study (data not are shown). The primers

(AG)8C produced 1-14 bands within 300 bp to 6 kb and (AG)8G displayed 2-10 bands within 350 bp to 3kb amplicon (Supplementary data). The dendrograms obtained using Unweighted Pair Group with Arithmetic Mean (UPGMA/Dice) with (AG)8C primer fingerprints formed five major clades (I to V) and 10 to 12 sub-clades, whereas (AG)8G primer fingerprints produced six major clades (I to VI) and 10 to 11 sub-clades that represents the high diversity among the *F. verticillioides* strains. The result suggests that there is random distribution of *F. verticillioides* strains in the geographic regions (Figure 3).

Furthermore, the statistical discriminatory power of both primers was tested using Simpson's (SID) and Shannon's (H) indices for measuring diversity by comparing the partitions online tool. SID was used to calculate the discriminatory potential of the various sets of primers from typing systems, and it indicated the probability of the different population data sets. Consequently, the SID index increases the discriminatory capacity of the typing technique. The confidence interval (CI) can be compared with the original Non-Approximated Confidence Interval (CINA). The SID and H indices data obtained after analysis were shown in Table 2. At 95% CI, SID and entropy H were resulted equivalent for both the primers.

Furthermore, (AG)8G specifies the microsatellite component better than those with the (AG)8 C fingerprint. The partition computations for each UPGMA/Dice dendrogram of the microsatellite component have been adjusted from 50 to 95%. The both (AG)8G and (AG)8C, the maximum coefficient value was determined to be 0.230 at a threshold of 65% (Fig 4). SID partitions value reported as 0.762 with (AG)8C at this threshold, 13 clusters found with SID values at these cutoffs. Similarly, (AG)8G generated 14 clusters with SID values of 0.785 for the partitions, and there was no significant difference between (AG)8G and (AG)8C, except for BIONCL-20, BIONCL-37, BIONCL-28, BIONCL-29, BIONCL-40, BIONCL-42, BIONCL-43, BIONCL-47, BIONCL-51, and BIONCL-56, which were classified into distinct clades.

The UPGMA dendrograms of (AG)8G fingerprints were more distinct than the (AG)8G examined based on clades and subclades. However, (AG)8G ISSR primer forms six major clades which are distributed nevertheless their geographic origin, but significantly correlated with the disease severity on maize. Clade I consist of more than 75% strains that showed low disease severity, except four strains originated from TN. The strains were obtained from MH showed moderate or high disease severity, clade II strains showed only moderate disease severity, whereas clades (III, IV, V) strains showed moderate or high disease severity except BIONCL 4 strain demonstrated low disease severity and three strains showed no disease severity. However, clade VI strains demonstrated low disease severity, except BIONCL 57 showed no disease severity (Figure 5). More comprehensive research, may necessitate for the genetic diversity of *F. verticillioides* in maize genotypes.

Discussion

The worldwide distribution of *Fusarium* species has rendered with high variability in its evolutionary characteristics. Apart from the fact, there is a lack of continuity in its taxonomy. Interestingly, *Fusarium* species were classified based on morphological observations revealed more than 1,000

species, strains, and forms [38]. Moreover, there is an extensive diversity among the unresolved *Fusarium* within the species [39]. The detailed and reliable classification system is still needed due to its high pathogenicity, and toxicity to plants and humans [40]. Most of the classifications within the species were based on the pathogenic behavior and vegetative compatibility groups (VGC) that aided imprecise identification of TEF1- α gene in diverse *Fusarium* species [41,42]. A total of 61 isolates were obtained, and 54 were belongs to the *F. verticillioides* [43]. *F. verticillioides* is a dominating species with a characteristic feature for producing a variety of mycotoxins in different food crops [11]. However, there were no extensive studies conducted on molecular diversity of *F. verticillioides* in India. Also, these species infect and contaminate different food crops viz., paddy, sorghum, small cereal grains, poultry, and cattle feed during the post-harvest period and produce various mycotoxins. Recently, Deepa et al. [44] reported 74% of *F. verticillioides* strains were associated with various food samples from Karnataka, India, in which 42% of strains showed positive signals for FUM production. In addition to its toxicity, *F. verticillioides* can cause the stalk corn rot in maize cultivars in Assam, India [45] and even resistant maize cultivar [46].

We have isolated, *F. coffeatum*, *F. foetens*, and *F. euwallaceae* in addition to *F. verticillioides*. *F. verticillioides* have been considered a plant pathogen belonging to FFSC, known to cause head, stalk, and root rots in maize due to the active production of FUM [47]. *F. coffeatum* belonged to the *Fusarium incarnatum-equiseti species complex* (FIESC), whereas *F. foetens* is a sister taxon of the *Fusarium oxysporum species complex* (FOSC) and a well-known plant pathogen that infect various cereals, including maize and ornamental crops [48-50]. *F. euwallaceae*, a symbiotic fungus of *Euwallacea* sp., was identified in an invasive ambrosia beetle from Israel and California, belongs to the *Fusarium solani species complex* (FSSC), showing the distinctive ecologies and exclusive lineage within Clade 3 of the FSSC [51]. To the best of our knowledge, there were no reports available either of the species, *F. coffeatum*, *F. euwallaceae* and *F. foetens* associated with food grain samples from India [52]. In the present study, for the first time, we reported these novel species, isolated from stored maize samples and their ability to produce FB1.

In addition to the precise identification of the species, molecular detection of mycotoxins biosynthetic pathway genes are utmost important for evaluating the toxigenic fungi to ensure the quality of the food. The traditional approaches are labor intensive and time consuming. Hence, the molecular methods have become a robust alternative due to their flexibility, precision, and low time requirements compared to the conventional methods. The developed PCR assay was able to detect FB1 from the culture and maize grains due to the high sensitivity and specificity of the primers [11].

In the present study, 54 isolates of *F. verticillioides* were evaluated for their genetic diversity study using ISSR fingerprints [53]. The ISSR fingerprints recognized the six clades and about 50% of the diversity within *F. verticillioides*. The microsatellite DNA formed several repetitive motifs that demonstrated substantial distinction among isolates. Moreover, no correlation was observed between the clustering within the *F. verticillioides* respective to geographical regions. Nevertheless, (AG)⁸G fingerprint formed six major clades, which showed a strong correlation with pathogenicity [54, 55]. Interestingly, Leyva-

Madrigal et al. [56] studied the polymorphic nature of ISSR markers, and 470 microsatellites were observed in the genome of *F. verticillioides*. ISSR fingerprints facilitated the studies on the relationships between *F. verticillioides* and showed SID ranged from 0.034 to 0.78 with both the fingerprints. The (AG)₈C and (AG)₈G markers revealed 252 and 368 microsatellites sites, respectively. About 90% of *F. verticillioides* enabled the produce FB1 which are isolated from the maize [55]. In the recent past, the diversity in *Fusarium* led to the description of several new specific and infra-specific taxa by examining the large populations. The association mapping using diverse genotypes was a promising and effective approach to gain useful insights that determined the functional variability of both known and unknown genes [57]. On the other hand, *Fusarium* species were profiled for toxigenic potentials using HPLC/LC-MS. About 31 strains showed moderate to high pathogenicity, on growth of the maize. The FB1 produced by *F. verticillioides* might be a virulence factors in disease development [58]. In addition, these species were known to possess virulent genes, which translated to the pathogenic proteins, thereby causing plant pathogenesis. However, there is a need to prove this hypothesis through mechanistic studies, determining the exact pathogenicity factors [59].

Conclusion

The molecular characterization of toxigenicity and pathogenicity of *F. verticillioides* strains associated with the stored maize grains from poultry industries in India was studied. *F. coffeatum*, *F. euwallaceae* and *F. foetens* were the first time reported from maize grain, India which produce FB1. The current observation revealed the polymorphic nature of seed borne *F. verticillioides* exhibited wide genetic diversity, severe pathogenic to maize studied by ISSR fingerprint. The (AG)₈G molecular marker could be efficiently used to precise identification and characterization of pathogenic *F. verticillioides* populations. Furthermore, the ISSR fingerprint clearly segregate the pathogenic and non-pathogenic clades in the *F. verticillioides* irrespective of the geographical origins. The identified and characterized molecular markers are more imperative for developing a disease-resistant crop breeding programme, which could lead to effective control of *Fusarium* pathogen. These findings might helps to understand the molecular mechanisms of host pathogen interaction and virulence of the pathogen to propose disease management policies based on molecular markers.

Declarations

The Author's declares that they don't have no competing Interests

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Authors' contributions

Vishwambar persuaded the concept and drafted the manuscript.

Dr.V. Koteswara Rao, conceptualization, scientific correction, and English editing of the manuscript.

Code availability:

Not Applicable

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Tables

Table 1

In -vitro pathogenicity of *Fusarium* species on Maize

Isolates code	<i>Fusarium</i> species	Seed germination inhibition (%)	Root inhibition (%)	Shoot inhibition (%)
BIONCL-1	<i>F.verticillioides</i>	40	8.55	8.24
BIONCL-2	<i>F.verticillioides</i>	40	19.81	28.35
BIONCL-4	<i>F.verticillioides</i>	30	19.81	5.67
BIONCL-6	<i>F.verticillioides</i>	60	4.05	19.58
BIONCL-7	<i>F.verticillioides</i>	10	16.66	21.13
BIONCL-8	<i>F.verticillioides</i>	70	10.81	8.76
BIONCL-9	<i>F.verticillioides</i>	20	11.71	1.28
BIONCL-10	<i>F.verticillioides</i>	80	12.16	7.41
BIONCL-11	<i>F.verticillioides</i>	30	9.00	6.28
BIONCL-12	<i>F.verticillioides</i>	20	6.75	7.21
BIONCL-14	<i>F.verticillioides</i>	20	40.09	32.98
BIONCL-16	<i>F.verticillioides</i>	40	23.42	21.13
BIONCL-17	<i>F.verticillioides</i>	70	47.29	46.39
BIONCL-18	<i>F.verticillioides</i>	70	35.58	20.61
BIONCL-19	<i>F.verticillioides</i>	90	25.67	28.35
BIONCL-20	<i>F.verticillioides</i>	40	25.67	28.35
BIONCL-22	<i>F.verticillioides</i>	60	7.20	9.02
BIONCL-23	<i>F.verticillioides</i>	70	20.04	21.90
BIONCL-24	<i>F.verticillioides</i>	90	2.25	15.46
BIONCL-25	<i>F.verticillioides</i>	70	8.10	1.54
BIONCL-26	<i>F.verticillioides</i>	90	2.70	3.09
BIONCL-27	<i>F.verticillioides</i>	70	13.96	17.52
BIONCL-28	<i>F.verticillioides</i>	60	4.95	1.54
BIONCL-29	<i>F.verticillioides</i>	20	24.77	21.13
BIONCL-30	<i>F.verticillioides</i>	70	22.07	19.58
BIONCL-31	<i>F.verticillioides</i>	80	50.90	30.41

BIONCL-32	<i>F.verticillioides</i>	40	42.34	35.56
BIONCL-33	<i>F.verticillioides</i>	00	20.72	11.81
BIONCL-34	<i>F.verticillioides</i>	90	0.00	21.64
BIONCL-35	<i>F.verticillioides</i>	80	21.59	17.11
BIONCL-37	<i>F.verticillioides</i>	60	65.31	56.70
BIONCL-38	<i>F.verticillioides</i>	80	30.63	37.62
BIONCL-39	<i>F.verticillioides</i>	90	16.66	38.14
BIONCL-40	<i>F.verticillioides</i>	50	67.11	59.79
BIONCL-42	<i>F.verticillioides</i>	80	72.07	62.82
BIONCL-43	<i>F.verticillioides</i>	90	32.8	36.08
BIONCL-44	<i>F.verticillioides</i>	60	34.23	32.98
BIONCL-45	<i>F.verticillioides</i>	40	43.24	39.17
BIONCL-47	<i>F.verticillioides</i>	90	15.76	28.35
BIONCL-48	<i>F.verticillioides</i>	20	25.67	39.17
BIONCL-49	<i>F.verticillioides</i>	90	82.43	71.64
BIONCL-50	<i>F.verticillioides</i>	90	12.61	26.80
BIONCL-51	<i>F.verticillioides</i>	10	11.10	27.03
BIONCL-52	<i>F.verticillioides</i>	70	8.56	11.10
BIONCL-53	<i>F.verticillioides</i>	NA	NA	NA
BIONCL-54	<i>F.verticillioides</i>	00	26.57	14.94
BIONCL-55	<i>F.verticillioides</i>	NA	NA	NA
BIONCL-56	<i>F.verticillioides</i>	50	6.78	28.04
BIONCL-57	<i>F.verticillioides</i>	30	NA	NA
BIONCL-58	<i>F.verticillioides</i>	60	NA	NA
BIONCL-59	<i>F.verticillioides</i>	70	7.02	31.07
BIONCL-64	<i>F.verticillioides</i>	10	7.20	18.55
BIONCL-71	<i>F.verticillioides</i>	80	4.95	5.67
BIONCL-75	<i>F.verticillioides</i>	80	28.37	38.14
BIONCL-60	<i>F. foetens</i>	NA	66.21	56.70

BIONCL-65	<i>F. foetens</i>	80	26.12	19.07
BIONCL-67	<i>F. coffeatum</i>	60	19.81	15.97
BIONCL-68	<i>F. coffeatum</i>	80	60.81	49.48
BIONCL-69	<i>F. coffeatum</i>	70	71.17	74.22
BIONCL-72	<i>F. coffeatum</i>	60	13.51	40.20
BIONCL-73	<i>F. euwallaceae</i>	90	41.93	56.49
	Control	NA	NA	NA

Table 2				
Diversity study for two ISSR primers with Simpson's index of diversity (SID) and Jackknife pseudo-values at confidence interval of 95% and entropy (H)				
ISSR primer	# partitions	Simpson's ID	Jackknife pseudo-values CI (95%)	H
(AG) ₈ C	2	0.230	(0.088-0.372)	0.556
	2	0.283	(0.141-0.425)	0.650
	4	0.663	(0.593-0.733)	1.666
	4	0.760	(0.741-0.778)	1.987
	6	0.762	(0.700-0.824)	2.201
(AG) ₈ G	2	0.073	(-0.027-0.172)	0.229
	4	0.595	(0.489-0.702)	1.492
	3	0.579	(0.492-0.665)	1.352
	5	0.755	(0.705-0.806)	2.078
	7	0.785	(0.729-0.842)	2.364

Figures

Figure 2

Fusarium verticillioides species specific PCR assay by vert f1 and vert f2 . Lane M - 100bp DNA ladder, lane 1 to 11 *Fusarium verticillioides* isolates

Figure 3

Phylogenetic tree of *F. verticillioides* strains by ISSR analysis with (AG)₈C (808) primer and (AG)₈G (809) primers

Figure 4

Cluster analysis of *F. verticillioides* strains by ISSR analysis (AG)₈C (808) primer and (AG)₈G (809) primers

Figure 5

Phylogenetic analysis of *Fusarium verticillioides* strains based on (AG)8G ISSR primer and their pathogenicity distribution in maize

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