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Cultural and Molecular Diversity of *Fusarium pallidoroseum* (Cooke.) Sacc Isolates Causing Wilt in Mothbean (*Vigna aconitifolia*)

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ABSTRACT

Background: Mothbean is minor pulse crop grown in arid and semi-arid region. Mothbean wilt is one of the most important diseases directly contributing to the yield losses of the crop. It is caused by *Fusarium* which shows the great cultural, morphological and molecular diversity. Nowadays, the meagre information known mothbean wilt and pathogen causing it.

Methods: Present investigation was conducted during 2022-2023, the fixed plot survey was carried out in moth bean growing farmers' fields of Bharuch district. Eleven isolates of the pathogen were collected from selected villages of Bharuch district. The cultural and morphological characteristics of pathogen were studied under laboratory condition as per standard methods. The molecular variability of eleven isolates causing mothbean wilt was studied employing standard six ISSR primers.

Result: During this investigation, the pathogen causing mothbean wilt is new record from South Gujarat location. Eleven *Fusarium* isolates causing mothbean wilt was found under Bharuch district of Gujarat. The cultural, morphological and molecular characteristics of eleven isolates were studied during present work. The present investigation will explore detailed study of *Fusarium* isolates causing mothbean wilt and their geographical cultural and morphological characteristics along with molecular diversity.

Key words: *Fusarium*, ISSR markers, Mothbean, Variability, Wilt.

INTRODUCTION

The excessive reliance on major staple crops, climate change and degradation of cultivable land hinder agricultural sustainability, posing challenges to global food security and exacerbating rural poverty and malnutrition in developing nations. Thus, a shift towards alternative crops, specifically underutilized plant species, is crucial for enhancing crop diversity, resilience and nutritional security in the face of evolving pests and pathogens (Kanishka *et al*., 2023). Pulses are an integral part of many diets across the globe and they have great potential to improve human health (Kharte *et al*., 2022) and these crops are major source of protein in the vegetarian diet (Yadav *et al*., 2022). The moth bean (*Vigna aconitifolia* L.) is an underutilized legume crop belonging to the *Fabaceae* family, subfamily Papilionaceae. It is the one of the most drought tolerant *Kharif* crop species of pulse category (Kohakade *et al*., 2017). Moth bean is a multipurpose crop but mainly grown for food, feed, pasture and fodder (Sedani *et al*., 2021). The regular consumption of mothbean will reduce cardiac diseases, diabetes and obesity of human. Mothbean seed contain abundant antioxidant as well as polyphenol the level helpful for human health (Bhadkaria *et al*., 2022). It is known as orphan legume as well as superior nutritional quality legume which can be cultivated widely and has ability to grow across different eco-geographical regions of Asia particularly under the Indian subcontinent (Kanishka *et al*., 2023).

Vascular wilt caused by *Fusarium pallidoroseum* is common disease in moth bean. A pathogen's greater adaptability strongly suggests the possibility of variations. *Fusarium* is most destructive soil borne fungus resulting into plant wilt and difficult to manage (Singh *et al*., 2019). The monocropping, cultivation of common variety/hybrids/ genotypes leads to evolution in pathogen behaviour and infection. Pathogen develop physiological and genetical variation among itself than parent one (Baysal *et al*., 2013). Cultural, morphological and molecular variability in different pulses like chickpea, pigeon pea, lentil grown areas is in reports (Thaware *et al*., 2017; Reddy *et al*., 2022). Variability is always a desirable attribute of a disease because it facilitates in the pathogen's survival in diverse niches and environments.The identification of variability helps in

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facilitating the development of effective strategies for managing multiple pathogenic variants (Arya *et al.,* 2021). Hence, an investigation was conducted to study the cultural, morphological and molecular variability among the isolates of *Fusarium* spp. in moth bean growing areas of Bharuch district.

MATERIALS AND METHODS

Isolation and pathogenicity of pathogen

The fixed plot survey was conducted during 2022 to study status of mothbean wilt under Bharuch district. The disease samples were collected from various locations of Vagra and Bharuch talukas of moth bean cultivating farmer's field as per mentioned in Table 1. The collected samples were bought to the laboratory at Department of Plant Pathology, College of Agriculture, Navsari Agricultural University, Bharuch. The isolation of pathogen was done by tissue isolation method and was further purified by single spore isolation technique. The isolated pathogen of each location samples were consider as isolates during study. The Koch postulates of isolated culture causing moth bean wilt was proved by growing moth bean plants in pots containing pathogen-infested soil as per (Patel, 2022).

Cultural and morphological variability

It is essential to understand cultural and morphological variation among various isolates for better understanding of molecular variability of *Fusarium* causing moth bean wilt The *Fusarium* isolates causing mothbean wilt were used to study cultural variation on PDA medium. The cultural and morphological study conducted in Department of Plant Pathology, College of Agriculture, NAU, Bharuch, Gujarat in 2022. The cultural characters like mycelium colour, texture, margin, pigmentation and radial growth were observed. Euromex microscope (Made in Netherlands) was used for conidial measurements. Total ten observations of each character *i.e*. width of mycelium, size of conidia, conidial measurement and numbers of septa in macro and micro-conidia, chlamydospores size and position were recorded for were recorded and average of each character was calculated.

Molecular variability

Eleven isolates of *Fusarium* inciting mothbean wilt were molecularly characterized during March-2023 by using various six ISSR primers (Table 3) synthesized by Barcode Biosciences Pvt. Ltd. The experiment was conducted in the laboratory of Plant Pathology, College of Agriculture, Navsari Agricultural University, Bharuch. The procedure for fungal DNA isolation was followed as per (Murray and Thompson, 1980) which were modified in annealing temperature and primer concentrations. Amplification of DNA during PCR reactions using ISSR primers was accomplished by using reaction mixture. [Template-2 µl (~50 ng of DNA), Primer-1 µl (~2.5 pmol), Master mix-7 µl, 10 picomole/µl oligonucleotide ISSR primers, 5U/µl Taq DNA polymerase, 10 X Taq buffer, 25 mM $MgCl_2$, 10 mM DNTP. The PCR amplification of DNA for ISSR markers werecompleted in 35 cycles (Denaturation at 95°C for 30 sec, Annealing at 50°C for 30 sec and Extension at 72°C for 1 min). The analysis of the recorded data was done by counting of the reproducible and clear bands with binary code 1 and 0. Jaccard's Similarity coefficient was generated using presence and absence of polymorphic bands with the use of NTSYS 2.20 Software. Based onsimilarity coefficient dendrogram was made by UPGMA (Unweighted Pair-Group Method with Arithmetical Averages). Jaccard's similarity coefficient was calculated as per standard formula and Polymorphic information content (PIC) was calculated as per (Kumar *et al.,* 2003).

RESULTS AND DISCUSSION

Isolation and pathogenicity of pathogen

The isolation of pathogen was done by tissue isolation method and the Koch postulates of isolated culture causing moth bean wilt were proved in pot condition. The pot inoculated with pathogen recorded typical mothbean wilt symptoms i.e. drooping of leaves and cortical decay of seedling, the leaves turned yellow followed by wrinkling and drying, loss of turgidity with brownish discoloration of vascular system. The plant dies after seven days of pathogen inoculation. The results are similar with Patel (2022) found wilting of mothbean after seven days of pathogen inoculation.

Cultural and morphological characteristics

The variability of pathogen was studied by morphological and cultural basis for better understanding of molecular variability of *Fusarium* causing moth bean wilt. Total eleven isolates of *Fusarium* causing moth bean wilt were collected and isolated from Vagra and Bharuch talukas of Bharuch district (Table 1).

The cultural studies based on the mycelium colour, mycelial texture, margin, pigmentation and radial growth showed huge variation in the cultural characteristics of the *Fusarium* isolates. The mycelium colour varied from dull whitish, pinkish white, purplish white, bright white and dull yellowish white. The mycelial texture varied from thick profuse, thin profuse, thin compact, moderately thin compact and moderately thin profuse (Table 1). Similar results are in accordance with Yadav *et al.* (2022) who observed cottony colours among different isolates of *F. verticillodies* in maize. The radial growth was observed maximum (50 × 50 mm) in isolate SAFM-4 and minimum (35 × 30 mm) in isolate ARFM-1 (Table 1). This results were corroborates with finding of Venkataramanamma *et al.* (2021) who found texture of the mycelium was fluffy, appressed, partially appressed, fluffy and thread like in nature. Saxena *et al.* (2019) observed yellowish pigmentation and dark brown with creamy as well as dark tan culture of *Fusarium oxysporum* f. sp. *lentis*.

The mycelial growth pattern was oppressed and fluffy. Purohit *et al.* (2017) observed that the colony diameter ranged from 2.8-9.7 cm.

The morphological studies based on size and breadth of conidia, number of septa in macro and micro-conidia and position of chlamydospores exhibited wide range of variation. The size of macro conidia of *Fusarium* isolates was ranged from 11.45 × 3.56 µm (BHFM-7) to 27.13 × 5.35 µm (BHFM-3) whereas in micro-conidia it was ranged from 7.23 × 3.02 µm (VAFM-6) to 15.59 × 5.14 µm (SA1FM-2) (Table 2). Septation in macro conidia ranged from 1-3 to 2-4. Isolate SA1FM-2 and RA2FM-5 recorded maximum (2-4) septation, followed by ARFM-1, BHFM-3, VAFM-6, BHFM-7, RA2FM-8, SA2FM-10 and ORFM-11 with (2-3) septation, tailed by SAFM-4 and ANFM-9 with (1-3) septation whereas in micro-conidia septation ranged from 0 to 1 (Table 2). The chlamydospores also showed remarkable variability in size and position of chlamydospores among the *Fusarium* isolates, the maximum (29.19 × 12.79 µm) was observed in isolate ORFM-11 and minimum (9.95 × 4.70 µm) in isolate BHFM-3 with the isolates showing either intercalary or terminal position (Table 2). Similar results are in accordance with

Table 1: The details of *Fusarium* isolates used during study and their cultural characteristics.

GPS Location	Code	Mycelium colour	Texture	Margin	Pigmentation	Radial growth (mm)
Lat- 21.793247° Long- 72.911314°	ARFM-1	White mycelium with center	Thin compact mycelium	Irregular	Dull whitish	35 × 30
Lat- 21.783951° Long- 72.834488°	SA1FM-2	Pinkish white	Thick profuse and Fluffy mycelium	Regular	Pinkish	49 × 47
Lat- 21.7737° Long- 72.8575°	BHFM-3	Bright white mycelium	Moderately thin compact mycelium	Regular	Dull whitish	48 × 47
Lat- 21.8071° Long- 72.8456°	SAFM-4	Dull white	Thin profuse mycelium	irregular	Dull whitish	50 × 50
Lat- 21.82883° Long- 72.889615°	RA2FM-5	Pinkish white	Thick profuse and Fluffy mycelium	regular	Pinkish white	46 × 44
Lat- 21.821345° Long- 72.837365°	VAFM-6	Dull white	Thick profuse mycelium	Irregular	Dull white with purple center	46 × 48
Lat- 21.777033° Long- 72.872154°	BHFM-7	Purplish white	Thick profuse mycelium	Regular	Light purplish white	49 × 47
Lat- 21.834193° Long- 72.883434°	RA1FM-8	Dull white yellow	Moderately thin profuse mycelium	Irregular	Dull white yellowish	48 × 49
Lat- 21.823478° Long- 72.883878°	ANFM-9	Dull white	Thin and compact mycelium	Irregular	Yellowish white	46 × 45
Lat- 21.783933° Long- 72.834328°	SA2FM-10	Bright white	Thick profuse fluffy mycelium	Regular	Light pinkish white	44 × 46
Lat- 21.834193° Long- 72.883434°	ORFM-11	Dull yellowish white	Thick profuse mycelium	Regular	Dull yellowish	48 × 49

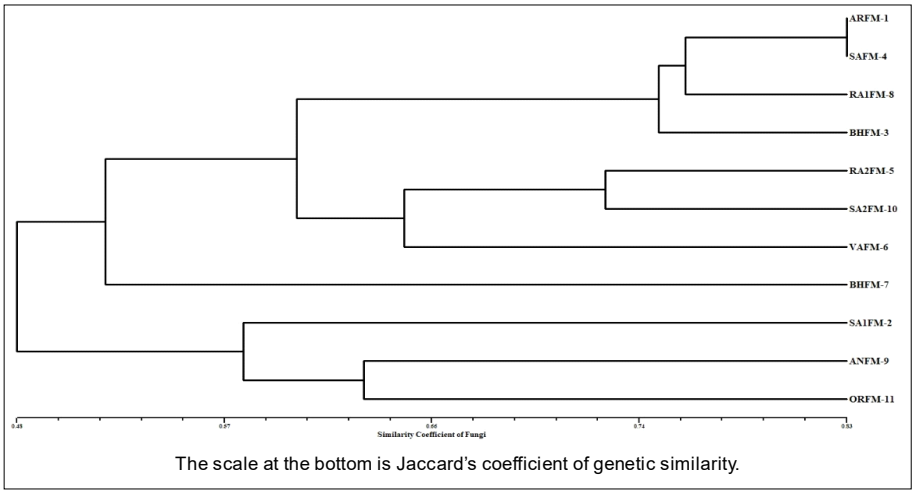
Table 2: Morphological characteristics of *Fusarium* isolates causing moth bean wilt.

Isolates	Macro conidia			Micro-conidia			Chlamydospores	
	Mean length (µm)	Mean width (µm)	No. of septa	Mean length (µm)	Mean width (µm)	No. of septa	Size (µm)	Position
ARFM-1	25.55	3.11	2 -3	13.13	4.16	0	14.06 × 8.74	Terminal
SA1FM-2	21.41	5.10	2-4	15.59	5.14	0-1	25.54 × 13.94	Terminal
BHFM-3	27.13	5.35	2- 3	11.65	4.53	0	9.95 × 4.70	Intercalary
SAFM-4	26.08	5.31	1-3	13.59	4.67	0	18.35 × 5.54	Terminal
RA2FM-5	11.62	3.75	2-4	7.65	3.21	0	18.66 × 11.39	Terminal
VAFM-6	13.08	4.03	2-3	7.23	3.02	0	17.51 × 9.21	Intercalary
BHFM-7	11.45	3.56	2- 3	7.11	3.37	0	18.63 × 9.82	Intercalary
RA1FM-8	20.86	5.56	2- 3	10.45	3.49	0	18.78 × 11.24	Terminal
ANFM-9	20.87	4.04	1- 3	11.48	4.15	0	14.15 × 7.88	Terminal
SA2FM-10	22.21	5.20	2- 3	14.21	5.24	0	13.43 × 6.70	Terminal
ORFM-11	18.23	3.72	2- 3	12.26	4.21	0-1	29.19 × 12.79	Intercalary

Table 3: Per cent polymorphism observed in ISSR primers.

Primer	Primer sequence	Annealing temp. (°C)	Total no. of loci	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism (%)	PIC*
(CAG) 5	CAGCAGCAGCAGCAG	50	10	95	0	5	100	0.39
Primer 2	GACGACGACGACGAC	50	9	100	0	5	100	0.36
(GTG) 5	GTGGTGGTGGTGGTG	50	9	102	0	7	100	0.35
ISSR10	CACCACCACCACCAC	50	7	77	0	6	100	0.32
LC48	AGAGAGAGAGAGAGAGG	50	9	79	0	8	100	0.40
LC49	GAGAGAGAGAGAGAGAT	50	10	35	0	9	100	0.39
		Total	54	-	-	40	-	-
		Average	9	-	-	6.66	100	0.37

*PIC- Polymorphic information content.



The scale at the bottom is Jaccard's coefficient of genetic similarity.

Fig 1: Dendrogram depicting the classification of the 11 *Fusarium* isolates constructed using UPGMA method based on ISSR markers.

Reddy *et al.* (2022) who found that the size of the micro-conidia ranged from 5.37 µm × 2.44 µm (Fsp-24) to 13.58 µm × 3.33 µm (Fsp-32) and the size of the macro conidia ranged from 16.62 µm × 3.35 µm (Fsp-35) to 39.05 µm × 4.61 µm (Fsp-33) and Hami *et al.* (2021) who observed septation in macro and micro-conidia, 3-4 and 0-1 respectively.

Molecular variability

At the molecular level the 11 isolates of the pathogen with ISSR (6 primers) showed significant variation with 100 per cent polymorphism (Table 3). The amplified bands obtained were of various sizes, ranging from approximately 0.1 to 1.7 Kb. The amplicon size for (CAG) 5 ranged from 0.3 to 1.6 Kb. The minimum similarity coefficient obtained was 0.28 and maximum similarity coefficient obtained was 0.83 (Fig 1). The results of pairwise combination indicated the closest relationship between the isolates ARFM-1 and SAFM-4 *i.e.* 0.83 and the minimum similarity coefficient ormaximum diversity was observed in between isolates SAFM-4, BHFM-7 and ORFM-11 *i.e.* 0.28 with ISSR primers. The ISSR primers clustered 11 isolates at 48 per cent similarity level in the two major clusters (A and B) (Fig 1). Similar results are in accordance with Arya *et al.* (2021) who evaluated Twenty-two isolates at 62 per cent similarity level and were clustered into two major clusters (A and B), cluster A consisted nine isolates, further A and B clusters were divided into four sub-clusters.Clusters A consisted of eight isolates *i.e.* ARFM-1, (Aragama), SAFM-4 (Saran), RA1FM-8 (Rahad-I), BHFM-3 (Bhersam), RA2FM-5 (Rahad-II), SA2FM-10 (Sayakha-II), VAFM-6 (Vagra) and BHFM-7 (Bharuch) and cluster B included three isolates *i.e.* SA1FM-2 (Sayakha-I), ANFM-9 (Ankot) and ORFM-11 (Rahad-original). The results are similar with Navale *et al.* (2023) who evaluated the genetic diversity of *Fusarium* spp. in maize through twenty ISSR primers and five five major clades (I to V) and 10 to 12 sub-clades were generated during study and Nawade *et al.* (2017) studied molecular diversity of *F. oxysporum* f. sp. *cumini* by 11 ISSR primers and it categorized in to two main clusters.

CONCLUSION

The pure culture of pathogen causing mothbean wilt was sent for the identification to Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi and it was identified as *Fusarium pallidoroseum* (Cooke.) Sacc (ITCC ID no. 11,584.21). Eleven isolates of *Fusarium* were isolated from moth bean growing areas of Bharuch district showed the maximum cultural, morphological and genetic variability

within isolates. The maximum (50 × 50 mm) radial growth was observed in isolate SAFM-4 and minimum (35 × 30 mm) in isolate ARFM-1. The size of macro conidia of *Fusarium* isolates was ranged from 11.45 × 3.56 µm (BHFM-7) to 27.13 × 5.35 µm (BHFM-3) whereas in micro-conidia it was ranged from 7.23 × 3.02 µm (VAFM-6) to 15.59 × 5.14 µm (SA1FM-2). The molecular variability of 11 isolates of *Fusarium* causing mothbean wilt was studied by ISSR primers which showed significant variation with 100 per cent polymorphism.

Conflict of interest

All the authors declared that there is no conflict of interest.

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