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MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS OF DIFFERENT RACES OF *Fusarium oxysporum* f.sp. *melonis* IN MUSKMELON USING RAPD MARKERS UNDER PUNJAB CONDITIONS

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ABSTRACT

Muskmelon wilt caused by *Fusarium oxysporum* f.sp. *melonis* causes serious damage in muskmelon growing districts of Punjab. Keeping in view the importance of disease, the present study was conducted with an objective to identify the different races of muskmelon wilt using molecular techniques. Twenty isolates of *F. oxysporum* f.sp. *melonis* were collected in 2018 and 2019 from different locations of muskmelon growing areas of Punjab viz. Jalandhar (J) Kapurthala (K), Sangrur (S) and Patiala (P). Pathogenicity for the evaluation of prevalent races of *F. oxysporum* f.sp. *melonis* under Punjab conditions was performed on a set of five differentials viz. Charentais T, Charentais FOM-1, Charentais FOM-2, Margot and Isabelle under pot house conditions. After 2-3 true leaf stage, these differentials were artificially inoculated with spore suspension @ 1×10^6 /ml of twenty isolates of *F. oxysporum* f.sp. *melonis*. Findings indicated that out of total 20 isolates, 8 isolates belonged to race 1, 8 isolates belonged to race 1.2, 3 isolates belonged to race 2 and remaining 1 belonged to race 0. Therefore, race 1 and 1.2 were prevalent in Punjab. Genetic diversity among the twenty isolates of *F. oxysporum f.sp. melonis* was analyzed with the Random Amplified Polymorphic DNA (RAPD) markers. Based on cluster analysis, J2 and J4 isolates were outliers and they were genetically different from other isolates. It was concluded that Fusarium wilt of muskmelon caused by FOM was prevalent in all muskmelon growing districts of Punjab. These findings are considerably important and could be used for selection and integration of muskmelon wilt resistance gene into muskmelon breeding programmes for Fusarium wilt management under Punjab conditions.

Keywords: Differential, *F. oxysporum* f.sp. *melonis*, Muskmelon wilt, Punjab, Races, RAPD markers

Fusarium wilt of melon (*Cucumis melo* L.), caused by *Fusarium oxysporum* f.sp.*melonis* Synd. and Hansen leads to significant yield losses or crop failures globally. *Fusarium oxysporum* f.sp. *melonis* is one of the most destructive pathogen in muskmelon (Banihashemi, 2010). Members of this form have an ability to specifically attack muskmelon crop and not other melons such as watermelon, squash melon or any other related cucurbits. In the recent years, continuous expansion of *Fusarium* wilt severely affected the quality and performance of melons (Gao *et al*., 2015).

Different pathotypes were classified on the basis of the variations prevailing in their virulence. To identify the most susceptible host *formae specialis* concept with the identification of pathogenic races has been widely accepted. The *F. oxysporum* f.sp. *melonis* has four races and all of these races are known to produce infection on susceptible genotypes. Soil-borne diseases have been intensified due to the incessant cultivation of susceptible melons and forced extensive use of chemicals (Park *et al.*, 2013). FOM race 1.2 has been further classified into two pathotypes which are 1.2Y and 1.2W that caused yellowing and wilting type symptoms, respectively (Chikh-Rouhou*et al.,* 2011).

Various methods used to detect *Fusarium oxysporum* f.sp. *melonis* include symptom development, spores, mycelium, and isolating fungus on selective medium. Since the growth of *Fusarium oxysporum* f.sp. *melonis* on the selective medium is slow and tentative, molecular investigations based on DNA techniques are considered to be one of the precise and rapid method (Oumouloud *et al.,* 2010). In *Fusarium oxysporum* f.sp.*melonis*, development of new races is mainly due to horizontal transfer of mobile genetic element and point mutations (Inami *et al.,* 2012). Different races of pathogen can be identified based on DNA sequence that are related to host specificity whether pathogenic or non-pathogenic (Luongo *et al.,* 2012).

The four distinct races (0, 1, 2, 1.2) of *Fusarium oxysporum* f.sp. *melonis* were identified and confirmed by inoculating them on differential host cultivars of muskmelon namely, Charentais T, Doublon, CM-17187. These races were defined by the resistance of host

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genes overcome by different variants of the pathogen (Risser *et al.,* 1976).

Furthermore, in case of *Fusarium oxysporum* molecular techniques using RAPD and translation elongation factor-1a (TEF-1a) sequences were commonly used to identify the races prevalent within different formae specialis and for the phylogenetic analysis of Fom races (Luongo *et al*., 2012). A specific identification of race 2 was done by the development of SCAR (Sequence Characterize Amplified Region). These markers were developed using recombinant technologies from RAPD studies, by excising RAPD fragment of 420bp (Ahmad *et al*., 2020). All these studies have been proved to be helpful in differentiation between and within the species group (Luongo *et al*., 2012).

Keeping in mind the role of prevailing race of the pathogen in the disease development at a particular location this work was aimed to provide an information on race spectrum of this pathogen on muskmelon crop under Punjab conditions, as presently no information is available on the pathogenic races of *Fusarium oxysporum* f.sp. *melonis* prevalent in Punjab. Also, this study envisages the genetic relatedness among FOM isolates by race determination and performed phylogenetic analyses of identified FOM races.

MATERIALS AND METHODS

Identification of FOM races on muskmelon differentials

To identify the prevalence of pathogenic races under Punjab condition, 20 isolates were collected from different locations of Punjab in year 2018 and 2019 (latitudes 29.30° North to 32.32° North and longitudes 73.55° East to 76.50° East 300m above mean sea level) viz. Patiala (P), Sangrur (S), Jalandhar (J) and Kapurthala (K). The collected isolates were then inoculated on differential hosts viz., Charentais T, Charentais FOM-1, Charentais FOM-2, Isabelle and Margot. These differential hosts were designated as resistant or susceptible to different known races based on their disease reaction (Table 1). Seeds of these differential hosts were procured from Hyderabad based VNR seed company. Differential hosts set of plant varieties differ from each other with a different single resistant gene, which can be distinguished by their qualitative differences in reaction to different pathogen strains i.e., difference in resistance and susceptible reactions (Luongo *et al*., 2012; Herman and Perl Treves, 2007).

Raising of seedlings of differentials

For raising seedlings of differentials, 96 wells plug trays were used. Seeds were sown in a mixture of coco peat, perlite and vermiculite in the ratio of 3:1:1, respectively. Each well of the plug tray was filled with the mixture and kept for overnight to absorb moisture after sprinkling water over it. Next morning, one seed per plug was sown for each differential and total five trays were maintained one tray for each differential variety. Trays were covered with the plastic sheet during night to maintain the temperature of 27-30°C and removed in morning hours. The plants were watered twice a day to provide optimum moisture conditions. To protect these seedlings from frost the trays were again covered with polythene sheet in the night for first 2-3 days.

Inoculation of differential with *Fusarium oxysporum* f.sp. *melonis*

The seedlings were inoculated artificially with pathogen spore suspension after 15-20 days, when seedlings reached the three true leaf stage (3 weeks old seedlings). The spore suspension was prepared using the 7 days old fully grown fungus culture. For this, the fungus was first grown and maintained on Potato Dextrose Agar (PDA) medium slants. After 7 days of complete growth of fungus on PDA slants the spore suspension was prepared by using autoclaved distilled water and the concentration was adjusted to 1×10^6 /ml. Inoculation of pathogen on differential hosts was done using seedling dip method as described by Luongo *et al*. (2013) and Herman and Perl Treves (2007).

Table 1. List of differential host cultivars used for identification of races prevalent in Punjab

		Reaction to races			
Cultivar	Resistance genes	0	1	2	1.2
Charentais T	None	S	S	S	S
Charentais Fom-1	Fom-1	R	S	R	S
Charentais Fom-2	Fom-2	R	R	S	S
Margot	Fom-1 + Fom-2	R	R	R	S
Isabelle	Fom-1 + Fom-2 + Polygenic recessive	R	R	R	S

Molecular identification and phylogenetic analysis of races of FOM

DNA of 20 isolates has been extracted with the help of Cetyl Tri methyl Ammonium Bromide (CTAB) method according to Murray and Thompson (1980). Quantity of DNA was estimated by using Nanoquant plate of TECAN 2000. A set of 6 RAPD primers has been used for the amplification of 20 isolates of *Fusarium oxysporum* f.sp. *melonis.* Amplification of 20 samples of DNA was done in 96 wells PCR plate in Eppendorf Master Cycler ProS and Thermoseal has been used to cover 96 plate to prevent evaporation of PCR product. A standard Protocol for PCR was carried out for RAPD assays, starting the cycle with initial denaturation at 94°C for 2 min, denaturation at 94°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2 min and final extension at 72°C for 5 min (Luongo*et al*., 2012). PCR product obtained was then visualized in 1.5% of Agarose gel under UV light (Luongo *et al*., 2012) and photographs were taken with the 'GeneSnap' software programme for further studies.

Scoring of PCR product

Different number of alleles were identified in total 20 isolates using six decamar primers. Binary matrix has been used for the analysis of amplified bands and were recorded as 1 if band present or 0 if band was not present. Size of different alleles was determined by comparing them with a known marker of 100 bp, scoring was done by selecting an allele of different base pair ranging from 150bp to 2500bp from the 100 bp ladder. This allele was compared with the PCR amplification product of RAPD primers for 20 samples of FOM. Samples in which band was presented scored as 1 and samples in which band was absent scored as 0. PIC value has been used to express the value of polymorphism that has been shown by the different primers. This value was given by Botstein *et al.* (1980). This value of each primer is pivot on different number of alleles that are present and the distribution of their frequency. PIC value is similar to the 'gene diversity'. PIC value for each primer was calculated by the Nei (1987) formula.

$$\mathbf{PIC} = 1 - \sum^{n} \quad \mathrm{Pij}^2$$

P_{ij} = frequency of j^{th} allele in i^{th} primer.

n = summation extends over 'n' pattern.

Statistical analysis

Dice's coefficient has been used to estimate similarity between the 20 isolates on the basis of number of bands that were shared by them. Similarity index value has been done on the basis of Nei and Li (1979) coefficient of similarity (Dij)given as;

Dij=2a/ (2a+b+c)

'a' represents matched fragments and b and c represents unmatched fragments. By this method, similarity index has been calculated and dendrogram was prepared with the help of UPGMA which is present in NTSYS.

RESULTS AND DISCUSSION

Identification of *Fusarium oxysporum* f.sp. *melonis* races on muskmelon differentials

Set of 5 differential lines was used to prove the pathogenicity and confirmation for the races of *Fusarium oxysporum* f.sp. *melonis*. Selected differential hosts helped in the identification of the different pathogen races on the basis of wilted or non-wilted response of the plants. Differential hosts had genes that showed susceptible and resistant reaction to some races. All the isolates showed their response on each differential host (wilted or non-wilted) after the period of 14 days. All plants of Charentais T differential were found to be wilted. Based on the plant reaction towards the pathogen race it was found that Charentais FOM-2 differentials showed wilted reaction to some isolates and non-wilted to other isolates. Whereas, both Isabelle and Magrot differentials showed non-wilted response towards all the isolates (Table 2).

On the basis of reaction of each isolate on five differentials namely Charentais T, Charentais FOM-1, Charentais FOM-2, Isabelle and Magrot races were confirmed against the twenty isolates collected from different muskmelon growing districts of Punjab (Table 3). Results obtained further indicated that isolate P1, K1 and K2 were highly virulent in nature as they produce wilting symptoms on differential Charentais T earlier among all the 20 isolates on 4^{th} day of inoculation.

The isolates were then categorized into four different groups based on races that are identified on the basis of disease reaction of different isolates on 5 differential hosts (Table 4). Group 1 has eight numbers of isolates that belong to race 1. Similarly Group 2 also has eight numbers of isolates that belong to race 1.2, Group 3 has only 1 isolate that belong to race 0 and Group 4 has three isolates belonging to race 2.

Variations were also observed within the isolates collected from one district as two isolates from Kapurthala (K) district belong to Group 1 race and four isolates belong to Group 2 race. Similarly, it has been found that two isolates of Patiala (P) district belong to Group 1 and two isolates belong to group 2. Large variations were found among the isolates of Sangrur (S) in which two isolates belong to Group 1, one isolate to Group 2,

Table 2. Reaction of plants with symptoms of fusarium wilt for five differential muskmelon cultivars inoculated with isolates of *Fusarium oxysporum* f.sp. *melonis* collected from different 20 locations of Punjab in 2018. {P=Patiala, K=Kapurthala, J=Jalandhar and S=Sangrur}

Isolate No.	Designation	Charentais T	Charentais Fom-1	Charentais Fom-2	Isabelle	Margot
1	P1	Wilted	Wilted	Non-wilted	Non-wilted	Non-wilted
2	P2	Wilted	Wilted	Non-wilted	Non-wilted	Non-wilted
3	P3	Wilted	Wilted	Wilted	Non-wilted	Non-wilted
4	P4	Wilted	Wilted	Wilted	Non-wilted	Non-wilted
5	J1	Wilted	Wilted	Wilted	Non-wilted	Non-wilted
6	J2	Wilted	Wilted	Non-wilted	Non-wilted	Non-wilted
7	J3	Wilted	Wilted	Non-wilted	Non-wilted	Non-wilted
8	J4	Wilted	Non-wilted	Wilted	Non-wilted	Non-wilted
9	J5	Wilted	Non-wilted	Wilted	Non-wilted	Non-wilted
10	S1	Wilted	Wilted	Non-wilted	Non-wilted	Non-wilted
11	S2	Wilted	Wilted	Wilted	Non-wilted	Non-wilted
12	S3	Wilted	Wilted	Non-wilted	Non-wilted	Non-wilted
13	S4	Wilted	Non-wilted	Non-wilted	Non-wilted	Non-wilted
14	S5	Wilted	Non-wilted	Wilted	Non-wilted	Non-wilted
15	K1	Wilted	Wilted	Wilted	Non-wilted	Non-wilted
16	K2	Wilted	Wilted	Wilted	Non-wilted	Non-wilted
17	K3	Wilted	Wilted	Wilted	Non-wilted	Non-wilted
18	K4	Wilted	Wilted	Wilted	Non-wilted	Non-wilted
19	K5	Wilted	Wilted	Non-wilted	Non-wilted	Non-wilted
20	K6	Wilted	Wilted	Non-wilted	Non-wilted	Non-wilted

one isolate to Group 3 and one isolate to Group 4. All groups of pathogen races were reported from Sangrur (S) district. In Jalandhar (J) district it was found that 2 isolates belong to Group 1 and one isolate belongs to Group 2 and two isolates belong to Group 4 (Table 4). All four races (0,1,2,1.2) of the pathogen were present all over the world but this study revealed that only race 1.2 and 1 are more prevalent under Punjab conditions. Out of 20, 16 isolates of *Fusarium oxysporum* f.sp. *melonis* that were collected from different muskmelon growing region of Punjab belonged to two races i.e., race 1 and race 1.2. Three isolates belonged to race 2 of FOM and only one isolate belonged to race 0 of *Fusarium oxysporum* f.sp. *melonis*.

Molecular identification and phylogenetic analysis of races of *Fusarium oxysporum* f.sp. *melonis*

RAPD analysis has been done for the phylogenetic analysis of *Fusarium oxysporum* f.sp. *melonis*. DNA of all 20 isolates was extracted and used for the analysis with RAPD markers. These markers were helpful to describe the genetic diversity among the 20 isolates from different locations of Punjab. Six random decamar primers were used and all the primers produced 1436 total number of bands across 20 isolates and produced multiple amplification of DNA that ranges from 150 to 2500bp.

Table 3. Confirmation of different races of 20 isolates by noting down their reaction on differential hosts {P=Patiala, K=Kapurthala, J=Jalandhar and S=Sangrur}

Isolate name	Races confirmed on differentials
P1	1
P2	1
P3	1.2
P4	1.2
J1	1.2
J2	1
J3	1
J4	2
J5	2
S1	1
S2	1.2
S3	1
S4	0
S5	2
K1	1.2
K2	1.2
K3	1. 2
K4	1. 2
K5	1
K6	1

Table 4. Categorization of 20 isolates on the basis of their races identified on set of 5 differential hosts

	Isolate name	Number of isolates	Race
Group 1	P1, P2, J2, J3, K5, K6, S1, S3	8	1
Group 2	P3, P4, J1, K1, K2, K3, K4, S2	8	1.2
Group 3	S4	1	0
Group 4	J4, J5, S5	3	2

Table 5. PIC value and number of alleles amplified by different primers used in RAPD analysis of different isolates of *Fusarium oxysporum* f.sp. *melonis*

Primer code	Sequence (5'-3')	PIC Value	Number of alleles amplified
F12	ACGGTACCAG	0.86	10
AN5	GGGTGCAGTT	0.92	17
N16	AAGCGACCTG	0.91	19
N19	GTCCGTACTG	0.93	19
F14	TGCTGCAGGT	0.95	18
F18	TTCCCGGGTT	0.89	13

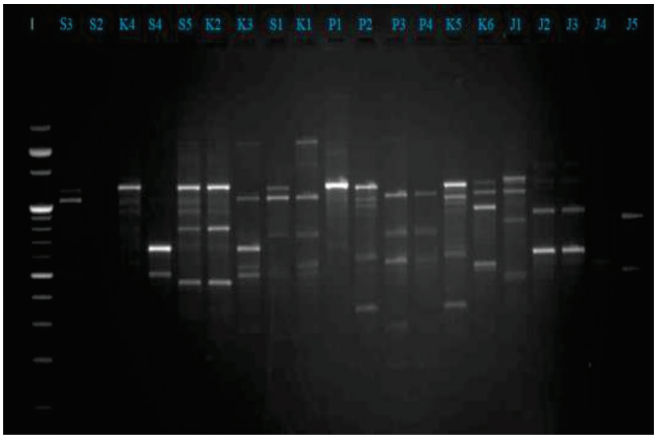

Fig. 1. *Fusarium oxysporum* f.sp. *melonis* (FOM) amplified with F-12 Primer (5' ACGGTACCAG 3')

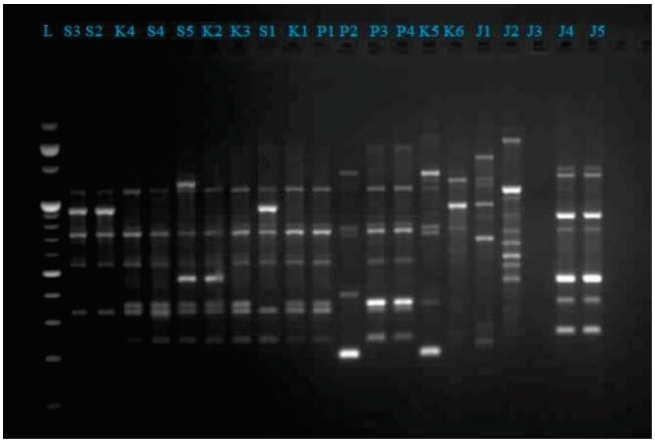

Fig. 2. *Fusarium oxysporum* f.sp. *melonis* (FOM) amplified with AN-5 Primer (5' GGGTGCAGTT 3')

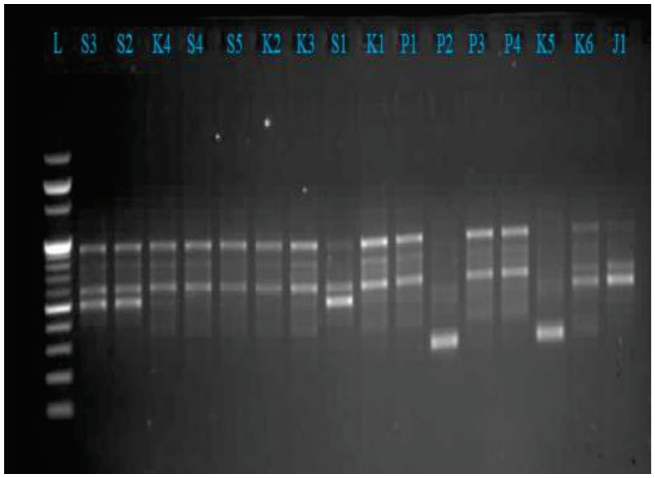

Fig. 3. *Fusarium oxysporum* f.sp. *melonis* (FOM) amplified with N-16 Primer (5' AAGCGACCTG 3')

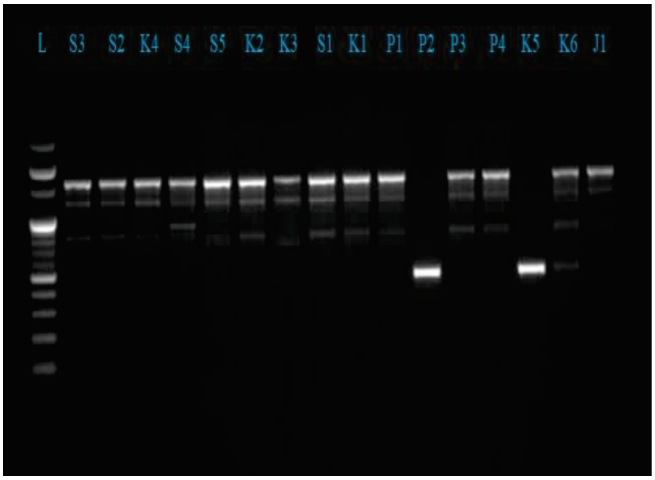

Fig. 4. *Fusarium oxysporum* f.sp. *melonis* (FOM) amplified with N-19 Primer (5¢ GTCCGTACTG 3¢)

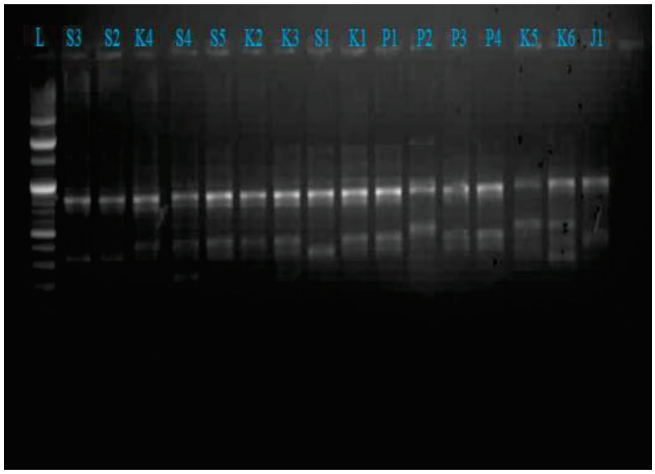

Fig. 5. *Fusarium oxysporum f.sp. melonis* (FOM) amplified with F-14 Primer (5¢ TGCTGCAGGT 3¢)

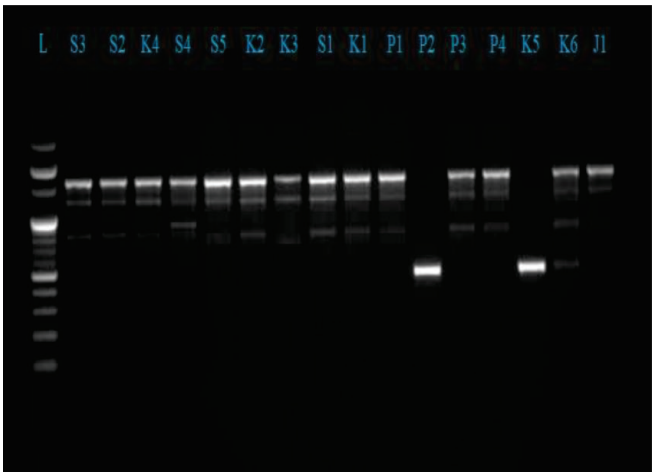

Fig. 6. *Fusarium oxysporum f.sp. melonis* (FOM) amplified with F-18 Primer (5¢ TTCCCGGGTT 3¢)

The PIC value of different primers ranged from. 0.86 in F-12 primer to 0.95 in F-14 primer. N-16 and AN-5 produce 137 and 99 bands, respectively and these primers were found to be highly polymorphic in nature having PIC value of 0.92 in N-16 and 0.93 in AN-5 (Table 5). Number of alleles amplified by the RAPD primers ranged from 10 to 19 maximum number of alleles amplified in N-16 and N-19 (19) followed by F-14 (18) whereas minimum number (10) of alleles was amplified by F-12 primer (Table 5).

Isolate J4 was identified using F-12 primer that differentiates it from the rest (Fig. 1). AN-5 and N-16 has been used to identify the K5, P1, J1 isolates as it produced the unique amplification products to these isolates (Fig. 2 and Fig. 3). P3 isolates can be differentiated by using N-19 primer and F-14 primer (Fig. 4 and Fig. 5). Similarly, primer F-18 differentiated P2 and K-5 isolate from the rest (Fig. 6).

Similarity matrix among the 20 isolates of *Fusarium oxysporum* f.sp. *melonis* from the amplification of DNA using RAPD markers was estimated by using Dice's coefficient of similarity (Sneath and Sokal, 1987). It was found that similarity coefficient ranged between 0.2 to 0.78 thus indicating high level of variability between these isolates (Table 6).

Cladogram of twenty *Fusarium oxysporum* f.sp. *melonis* (FOM) isolates was developed from the genetic distance based on random amplified polymorphic DNA (RAPD) and was generated by unweighted pair-group arithmetic mean method (UPGMA). J2 and J4 isolates of Jalandhar district were outliers and they were genetically different from other isolates of different districts of Punjab. Maximum genetic diversity was found between isolates P2 and J4 with coefficient value of 0.131. The isolates of *Fusarium oxysporum* f.sp. *melonis* from different muskmelon growing areas of Punjab were further divided into six groups on the basis of unweighted pair-group arithmetic mean method (UPGMA) analysis. Group 1 had two isolates belonging to Jalandhar district i.e., J3 and J5 with coefficient value of 0.381. Group 2 had one isolate belonging to Patiala district i.e., P2 and one belonged to Kapurthala district i.e., K5 with coefficient value of 0.507. Group 3 had one isolate belonging to Kapurthala district i.e., K6 and one belonged to Jalandhar district i.e., J1 with coefficient value of 0.455. Group 4 had two isolates that belonged to Patiala district i.e., P3 and P4 with coefficient value of 0.721. Group 5 had three isolates belonging to Sangrur district i.e., S2, S3 and S4 with coefficient value ranging from 0.553 to 0.688. Group 6 had highest number of

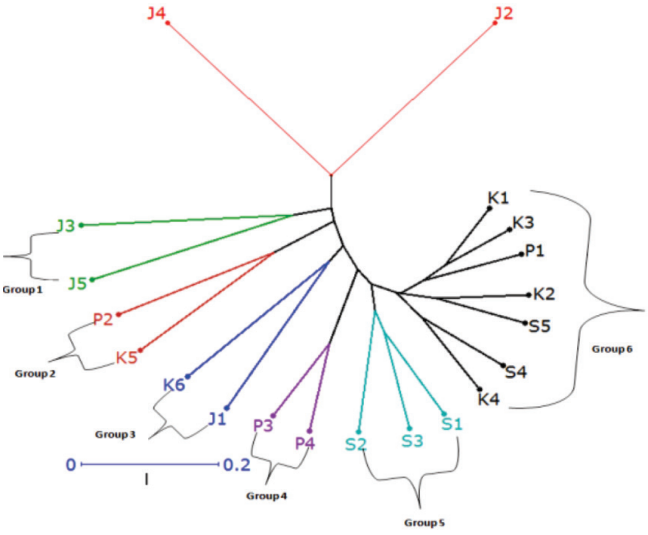

Fig. 7. Cladogram of twenty *Fusarium oxysporum* f.sp. *melonis* (FOM) isolates was developed from the genetic distance based on random amplified polymorphic. DNA (RAPD) and generated by unweighted arithmetic mean method (UPGMA)

Table 6. Similarity index by the 23 RAPD primers

Isolate	S3	S2	K4	S4	S5	K2	K3	S1	K1	P1	P2	P3	P4	K5	K6	J1	J2	J3	J4	J5
S3	1																			
S2	0.685	1																		
K4	0.620	0.628	1																	
S4	0.526	0.530	0.718	1																
S5	0.470	0.444	0.615	0.580	1															
K2	0.542	0.446	0.652	0.602	0.731	1														
K3	0.577	0.517	0.600	0.662	0.637	0.605	1													
S1	0.688	0.553	0.477	0.479	0.532	0.562	0.642	1												
K1	0.628	0.464	0.560	0.566	0.624	0.645	0.780	0.667	1											
P1	0.523	0.514	0.639	0.606	0.584	0.658	0.671	0.580	0.733	1										
P2	0.355	0.294	0.400	0.262	0.366	0.292	0.282	0.387	0.282	0.329	1									
P3	0.476	0.391	0.496	0.530	0.528	0.547	0.636	0.539	0.623	0.595	0.294	1								
P4	0.447	0.381	0.425	0.454	0.405	0.500	0.538	0.522	0.588	0.573	0.303	0.721	1							
K5	0.282	0.250	0.266	0.217	0.293	0.290	0.318	0.329	0.306	0.273	0.507	0.194	0.314	1						
K6	0.327	0.351	0.313	0.310	0.286	0.336	0.373	0.420	0.373	0.354	0.288	0.432	0.408	0.364	1					
J1	0.443	0.375	0.322	0.304	0.347	0.317	0.382	0.468	0.408	0.325	0.310	0.431	0.418	0.347	0.455	1				
J2	0.326	0.309	0.385	0.338	0.352	0.438	0.268	0.253	0.295	0.370	0.313	0.221	0.234	0.268	0.288	0.268	1			
J3	0.248	0.357	0.317	0.224	0.247	0.270	0.222	0.234	0.196	0.293	0.275	0.214	0.201	0.342	0.373	0.260	0.362	1		
J4	0.217	0.177	0.211	0.237	0.2	0.240	0.219	0.232	0.248	0.239	0.131	0.177	0.226	0.2	0.254	0.215	0.328	0.143	1	
J5	0.202	0.258	0.260	0.271	0.246	0.256	0.219	0.217	0.248	0.269	0.213	0.210	0.271	0.215	0.313	0.292	0.279	0.381	0.236	1

isolates i.e., seven. In this group, four isolates belonged to Kapurthala district i.e., K1, K2, K3 and K4, two belonged to Sangrur district i.e., S4 and S5 and one isolate belonged to Patiala i.e., P1 with coefficient value ranged from 0.580 to 0.780 (Table 6 and Fig. 7).

All the four races of the FOM were found to be prevalent throughout the different melon growing areas of world and known to cause disease on the susceptible cultivars. Different races of the pathogen were already categorized according to their virulent reaction on various differential hosts (Patel *et al*., 2017; Luongo *et al*., 2015; Kurt *et al*., 2002; Punja *et al*., 2001). There are also reports of generation of new pathotypes through mutation which are common in occurrence in *F. oxysporium* even though the evolution of a pathogen race is affected by host genotype (Gordon and Martyn, 1997). The most recent horizontal gene transfer evidences strongly supported the existence of both non-pathogenic and pathogenic strains in *F. oxysporum* (Fourie *et al*., 2011; Ma *et al*., 2010; Rep and Kistler, 2010). Random Amplified Polymorphic DNA (RAPD) analysis was used by some workers to divide different isolates of *Fusarium oxysporum* into four groups and they assigned Roman symbols to these groups. (Vakalounakis *et al*., 2005). Vast variations among the pathotypes have been observed by various researchers (Edel-Hermann and Lecomte, 2018; Majadh and Tuwarji, 2015; Chikh-Rouhou *et al*., 2011; Moretti *et al*. ,2002). Comparative use of genomic-based markers for the discrimination of host specificity in *Fusarium oxysporum* has been extensively studied by many scientists and is the next step to identify host specificity in *F. oxysporum* .The translation elongation factor 1-α (*TEF1*) and the DNA-directed RNA polymerase II largest subunit (*RPB1*) and second largest subunit (*RPB2*) are *Fusarium* phylogenetically informative *loci* and allow for species identification (O'Donnell *et al*., 2015). Whole genome sequencing of 45 *F. oxysporum* strains was performed and managed to differentiate *formae speciales cucumerinum*, *niveum*, *melonis*, *radicis-cucumerinum*, and *lycopersici* on the basis of their effector pattern (Van Dam *et al*., 2018, 2016).

In nutshell, the present work exhibited that Fusarium wilt of muskmelon caused by FOM was prevalent in all muskmelon growing districts of Punjab namely Jalandhar, Kapurthala, Patiala and Sangrur of Punjab region. All the four races of FOM i.e., 0, 1, 2 and 1.2 were reported under Punjab conditions. Further, the FOM isolates were divided into six group and two isolates namely, J2 and J4 were found to be highly genetically divergent from others as proved by the genetic diversity analysis with RAPD markers. Hence, this study could be considerably vital for the selection and integration of muskmelon wilt resistance gene into muskmelon breeding programmes for Fusarium wilt management.

Authors' contribution

Conceptualization of research work and designing of experiments (DSB, NS, SPS, MSH); Execution of lab/ field experiments and data collection (DSB and GS); Analysis of data and interpretation (DSB, GS, MSH, SPS); Preparation of manuscript (DSB, GS, SKA).

LITERATURE CITED

Ahmad M M, Babak R and Seyyed A K 2020. Identification of Fusarium wilt resistance sources in melon (*Cucumis melo* L.) landraces of Iran using marker-assisted selection technique. *Austral Plant Pathol* **49**: 413-23.

Banihashemi Z 2010. Reaction of Cucumis melo cultivars to races of *Fusarium oxysporum f.sp. melonis* the cause of melon vascular wilt. *Iran J Plant Pathol* **46**: 1.

Botstein D, White R D, Skolnick M and Davis R W 1980. Construction of genetic linkage map in man using restriction fragment length polymorphism. *Am J Hum Gen* **32**: 314-31.

Chikh-Rouhou H, Gonzalez-Torres R, Oumouloud A and Alvarez J M 2011. Inheritance of race 1.2 *Fusarium* wilt resistance in four melon cultivars. *Euphytica* **182**: 177-86.

Edel-Hermann V and Lecomte C 2018. Current status of *Fusarium oxysporum Formae speciales* and races. *Phytopathol* **109**: 512-30.

Fourie G, Steenkamp E T, Ploetz RC, Gordon T R and Viljoen A 2011. Current status of the taxonomic position of *Fusarium oxysporum formae specialis cubense* within the *Fusarium oxysporum* complex. *Infec Gen Evol* **11**: 533-42.

Gao P, Liu S, Zhu Q and Luan F 2015. Marker-assisted selection of Fusarium wilt-resistant and gynoecious melon (*Cucumis melo* L.). *Gen Mol Res* **14**: 16255-64.

Gordon T R and Martyn R D 1997. The evolutionary biology of *Fusarium oxysporum*. *Ann Rev Phyto Pathol* **35**: 111-128.

Herman R and Perl-Treves R 2007. Characterization and inheritance of a new source of resistance to *Fusarium oxysporum* f. sp. *Melonis* race 1.2 in *Cucumis melo*. *Plant Dis* **9**:1180-86.

Inami K, Yoshioka-Akiyama C, Morita Y, Yamasaki M, Teraoka T and Arie T 2012. A genetic mechanism for emergence of races in *Fusarium oxysporum* f.sp. *lycopersici*: Inactivation of avirulence gene AVR1 by transposon insertion. *PLoS ONE*.7: e44101.

Kurt S, Baran B, Sarı N and Yetisir H 2002.Physiologic races of *Fusarium oxysporum* f.sp. *melonis* in the southeastern anatolia region of turkey and varietal reactions to races of the pathogen. *Phytoparasitica*.**30**: 395-402.

Loungo L, Ferrarini A, Haegi A, Vitale S, Polverari A and Belisario A 2015. Genetic diversity and pathogenicity of *Fusarium oxysporum* f.sp. *melonis* races from different areas of Italy. *J Phytopathol* **163**:73-83.

Luongo L, Vitale S, Haegi A and Belisario A 2012. Development of SCAR markers and PCR assay for *Fusarium oxysporum* f. sp. *Melonis*race 2-specific detection. *J Pl Pathol* **94**: 193-99.

Ma L J, Van der Does H C and Borkovich K A 2010. Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium. *Nature* **464**: 367-73.

Majdah M Y and Al-Tuwaijri 2015. Studies on Fusarium wilt Disease of Cucumber. *J Appl Pharm Sci* **5***:*110-19.

Moretti A, Belisario A, Tafuri A, Ritieni A, Corazza L and Logrieco A 2002. Production of beauviricin by different races of *Fusarium oxysporum* f. sp. *melonis*, the Fusarium wilt agent of muskmelon. *Eur J Pl Pathol* **108**: 661-66.

Murray M G and Thompson W F 1980. Rapid isolation of high molecular weight plant DNA. *Nuc Acid Res* 8:4321-25.

Nei M and Li W H 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceed. *Nat Acad Sci USA* **76**:5269-73.

Nei M 1987. Molecular Evolutionary Genetics. Columbia University Press, New York. Pp. 645.

O'Dennell K, Ward T J, Robert V A R G, Crous P W, Gieser D M and Kang S. 2015. DNA sequence based identification of Fusarium: Current status and future directions. *Phytoparasitica* **43**: 583-95.

Oumouloud A, Arnedo-Andres M S, Gonzalez-Torres Rand Alvarez J M 2010. Inheritance of resistance to *Fusarium oxysporum* f. sp. *Melonis* races 0 and 2 in melon accession Tortuga. *Euphytica* **176**: 183-89.

Park D K, Son S H, Kim S, Lee W M, Lee H J, Choi H S, Yang E Y, Chae W B, Ko H C and Huh Y C 2013. Selection of melon genotypes with resistance to Fusarium wilt and Monosporascus root rot for rootstocks. *Plant Breed Biotech* **1**: 277-82.

Patel S A H, Vashisht V K and Dhatt A S 2017. Fusarium wilt of melon: Resistance breeding and gene development. *Kavaka* **49**:50-58.

Punja Z K, Parker M and Elmhirst 2001. Fusarium wilt of field grown muskmelon in British Columbia. *Can J Plant Pathol* **23**: 403-10*.*

Rep M and Kistler H C 2010. The genomic organization of plant pathogenicity in Fusarium species. *Curr Opi Plant Bio* **13**:420-26.

Risser G, Banihashimi Z and Davis D W1976. A proposed nomenclature of *Fusarium oxysporum*f.sp. *melonis*races and resistance genes in *Cucumis melo*. *Phytopathol* **66**: 1105-06.

Sneath P H A and Sokal R R 1973. *Numerical Taxonomy*: *The Principles and Practices of Numerical Classification*.W.H. Freeman and Co., San Francisco, USA. 573p.

Vakalounakis D J, Wang Z, Fragkiadakis G A, Skaracis G N and Li D B 2005. Characterization of *Fusarium oxysporum* isolates obtained from cucumber in China by pathogenicity, VCG, and RAPD. *Plant Dis* **88**:645-49.

Van Dam P, de Sain, M, ter Horst A, van der Gragt M and Rep M 2018. Use of comparative genomics-based markers for discrimination of host specificity in *Fusarium oxysporum*. *Appl Environ Microbiol* **84**:1868.

Van Dam P, Fokkens L, Schmidt S M, Linmans J HJ , Kistler H C, Ma L J and Rep M 2016. Effector profile distinguish *formae speciales* of *Fusarium oxysporum*. *Environ Microbiol* **18**: 4087-4102.