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Identification of Races of *Fusarium oxysporum* f.sp. *ciceris*, Inciting Wilt of Chickpea in Andhra Pradesh and Parts of Telangana

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ABSTRACT

Background: *Fusarium oxysporum* f.sp. *ciceris* (Foc) is one of the most important pathogen, causing wilt of chickpea. It is soil and seed borne pathogen. A serious threat in Foc is the evolution of new races, which reduces exploitation of wilt resistance in the crop in a particular area. Eight races (race 0, 1A, 1B/C, 2, 3, 4, 5 and 6) were reported in the world and among them four races (race 1, 2, 3, 4) were from India. Race 1 was reported from Andhra Pradesh. It is very important to monitor the variation regularly in new isolates collected from different varieties or genotypes and different geographical regions to identify the racial pattern. Development of SCAR markers for identification of Foc isolates is also important as they are highly reliable.

Methods: Twenty isolates of *Fusarium oxysporum* f.sp. *ciceris* were obtained from wilt infected plants of chickpea covering different places of Andhra Pradesh including two isolates from Telangana and confirmed the pathogen based on pathogenicity test. They were tested on host differentials of chickpea for races identification. Among these twenty isolates five most virulent isolates (Foc-6, Foc-10, Foc-12, Foc-17 and Foc-24) were selected for RAPD based on pathogenicity test and SCAR marker was developed based on DNA banding pattern during RAPD for one isolate.

Result: Based on the disease reaction on differentials, concluded that 17 of them out of 20 were matched with race-1 reaction. Two isolates were matched with race-6 reaction and one is not matched with none of the races. An amplified product of polymorphic band of approximately 700 bp in the isolate Foc-12, obtained during RAPD analysis was selected for SCAR marker development and two SCAR markers were developed and validated. Identification of races mainly helps in development of resistant cultivars to specific races and might be contributed to development of integrated disease management practices for Fusarium wilt.

Key words: Chickpea, Fusarium wilt, Race, RAPD, SCAR marker.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the important pulse crop grown in Andhra Pradesh as well as in India and second most important food legume after common bean in the world. In India, it is cultivated in an area of 9.67 million ha, with a production of 10.09 million tones, having productivity of 1043 kg/ha (Project Coordinators Report, 2019). In Andhra Pradesh, it is grown in an area of 5.2 lakh ha having a production of 5.88 lakh tones and recorded productivity of 1132 kg/ha during 2017-18. Several factors were responsible for yield losses of chickpea and it is reported that the crop is attacked by about 52 number of pathogens (Nene *et al*., 1984). Among the diseases, Fusarium wilt incited by *Fusarium oxysporum* f.sp. *ciceris* is one of the major constraint for production and causes losses by reducing the plant population. Under favourable agro climatic conditions, it caused yield losses in the range of 10 to 100% (Jendoubi *et al*., 2017). It is both seed and soil borne.

Existence of races is one of the important concern of *F. oxysporum* f.sp. *ciceris.* In the world, eight races (race 0, 1A, 1B/C, 2, 3, 4, 5 and 6) were reported and among them four races (race 1, 2, 3, 4) were from India (Haware and Nene, 1982). Two races *i.e*., race 0 and 1B/C induced yellowing symptoms (yellowing pathotype), whereas others caused wilting (wilting pathotype) (Jimenez-Diaz *et al.,* 1993). Among eight races, three races *i.e*., race 2, race 3 and race 4 are reported only from India. Race 1A is widely spread in India and also reported from Andhra Pradesh. A serious threat in Foc is the evolution of new races, which reduces exploitation of wilt resistance in the crop in a particular area. Therefore, it is essential of monitoring of variation regularly in new isolates collected from different varieties or genotypes and different geographical regions, which is the key to success in resistance breeding programme of Fusarium wilt.

Development of DNA based specific markers is required for pathogen identification, as they are more sensitive and precision in nature. Among the PCR based markers, SCARs have the advantage when compared to RAPD, due to its

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primer size and less sensitive to the standard PCR conditions and used for identification of isolates. Apart from that specificity is more and reproducible. Keeping all these points in view, this study was undertaken with the aim to identify races and development and validation of SCAR marker.

MATERIALS AND METHODS

Isolates of Foc were collected from wilt infected plants of chickpea from different places of A.P and part of Telangana state during 2014-15 and 2015-16. After isolation and morphological characterization, pathogenicity test was done to confirm it as Foc. Races identification was established based on the disease reaction of host differentials such as C-104, JG-74, CPS-1, BG-212, WR- 315, Chaffa, JG-62, GPF-2, DCP-92-3 and KWR-108 (Anonymous, 2015-16) and Annegiri, L-550 and K-850 (Anonymous, 2016-17) under protected conditions as per the procedure described by Dubey and Singh (2008). It was done for two consecutive years such as 2016 and 2017. About one week old pathogen was multiplied on sterilized sorghum grains. Each host differential of about ten seeds were sown in pots in three replications and control was also maintained without pathogen inoculation for comparison. Green house temperatures were maintained at 25°C to 30°C throughout the experiment and data was recorded starting from 15 days onwards at 15 days interval till plant gets wilted. Disease reaction was graded as resistant (0-20% wilt), moderately resistant (>20 to 50%) and susceptible (>50%) as per the scale of Haware and Nene, (1982).

Five most virulent isolates (Foc-6, Foc-10, Foc-12, Foc-17 and Foc-24), which recorded 100% wilt incidence on susceptible check (JG-62) were selected for RAPD to develop SCAR marker. The banding pattern among five isolates was observed with 15 RAPD primers (Table 1).

Amplification of DNA from different isolates was carried out in PCR (make Eppendorf) and 40 number of cycles were carried out. Initial denaturation was done at 94°C for 4 min, then denaturation at 94°C for 30 sec, followed by annealing at 37°C for 1 minute and extension is at 68°C for 1minute. Final extension was carried out at 68°C for 15 minutes.

Table 1: Random primers used for RAPD analysis.

Primer	Nucleotide sequence (5′ to 3′)
K1	TGCGTGCTTG
K2	ACTTCGCCAC
K3	GGCTCATGTG
K4	CAAACGTGGG
K5	CGAGGTCGACG
K6	CACCGCCCCAA
K7	GTCCTCAGTCCC
OPX-10	CCCTAGACTG
OPX-13	ACGGGAGCAA
P2	TACGGCTGGC
P8	CAGGCCCTTC
P17	TACGGCTGGC
P19	GCGGCATTGT
P21	CCAGACAAGC

Different banding pattern was obtained with different primers in RAPD. The amplified polymorphic band, which was unique was selected from one isolate and used for SCAR marker development. Then this band was eluted and cloned. After cloning, sequencing was done and after sequence alignment, by using Primer 3 (v.0.4.0) software (Rozen and Skaletsky, 2000) SCAR primers were designed and used for validation of that specific DNA band size. Genomic DNA amplification (1 ng/µl) with SCAR primers was done in a similar condition except annealing temperature, which was specific to each primer and finally amplification products were resolved electrophoretically in 1% agarose gel.

RESULTS AND DISCUSSION

Eighteen *Fusarium oxysporum* f. sp. *Cicer is* isolates from Andhra Pradesh and two isolates from Telangana were collected from wilt affected plants (Table 2). After isolation, the pathogen was identified as Foc based on cultural characters and morphological characters (micro and macro conidia and chlamydospores) (Venkataramanamma *et al*., 2019). All these isolates were confirmed as Foc based on pathogenicity test on susceptible check JG-62 (Table 2). These 20 isolates were subjected to virulence analysis on host differentials and all the isolates varied in causing wilt (0-100%) during the period. Incubation period differed from 16 to 40 days for wilting of host differentials for all Foc isolates. In more virulent isolates, the initial symptoms on JG-62 started to appear from 16th day onwards. It is in accordance with Durai *et al*. (2012), who reported that incubation phase for Foc isolates was 15-35 days, whereas virulent isolates cause wilting and death of seedlings in 11-15 days and 25% of the isolates from A.P., Gujrat, Uttar pradesh and Rajasthan induced seedling mortality in 16-20 days. In two isolates (Foc-14 and Foc-18) wilting symptoms first observed on chaffa rather than JG-62.

Among 20 isolates studied, 16 isolates showed susceptible, 3 isolates showed moderately susceptible reaction on JG-62. All isolates recorded reaction as resistant on differentials such as CPS-1, BG-212, WR-315, GPF-2 and moderate susceptible to resistant reaction on JG-74 and KWR-108 (Table 3 and 4). Whereas the cultivars C-104 and DCP-92-3 exhibited different reactions such as resistant, susceptible and moderate susceptible for Foc isolates. The cultivar chaffa exhibited moderate susceptible to susceptible reaction for isolates except Foc-3 and Foc-32. The two isolates (Foc-3 and Foc-32) which showed resistant reaction on chaffa were also examined on old host differentials *i.e*., Annegiri, L-550 and K-850 and found resistant reaction on Annegiri and K-850 cultivars. Among 20 isolates, 17 isolates were categorized into race-1 based on resistant reaction on CPS-1, BG-212 and GPF-2 (Fig 1). Two isolates such as Foc-3 (Kodumur, Kurnool dt, Andhra Pradesh) and Foc-32 (Bodhan, Nizamabad dt, Telangana) showed reaction as resistant on

Table 2: *Fusarium oxysporum* f.sp. *ciceris* isolates from different places of Andhra Pradesh and Telangana and pathogenicity test.

Name of the isolate	Place of collection	District	Pathogenecity test		
			Incubation period (days)	Wilt incidence (%)	Pathogenic virulence
Andhra Pradesh					
Foc-1	Yemmiganur	Kurnool	29	61.25	HP
Foc-3	Kodumur	Kurnool	30	67.30	HP
Foc-4	Nandyal	Kurnool	27	85.00	HP
Foc-6	Allagadda	Kurnool	20	100	HP
Foc-8	Ongole	Prakasam	27	86.50	HP
Foc-9	Addanki	Prakasam	35	45.00	MP
Foc-10	Marripudi	Prakasam	27	90.00	HP
Foc-12	Cumbum	Prakasam	20	100	HP
Foc-14	Parigi	Anantapuramu	26	78.00	HP
Foc-15	Kalyandurgam	Anantapuramu	30	60.00	HP
Foc-17	PD Palli	Anantapuramu	20	100	HP
Foc-19	Kaligiri	Nellore	38	13.30	WP
Foc-21	Vinjamur	Nellore	32	69.00	HP
Foc-22	Tullur	Guntur	30	65.10	HP
Foc-23	Tadikonda	Guntur	36	42.60	MP
Foc-24	Duttalur	Nellore	26	90.00	HP
Foc-26	Vempalli	Kadapa	35	50.00	MP
Foc-28	Proddutur	Kadapa	35	33.33	MP
Telangana state					
Foc-30	Adilabad	Adilabad	25	82.00	HP
Foc-32	Bodhan	Nizamabad	25	95.00	HP

HP: Highly pathogenic; MP: Moderately pathogenic; WP: Weakly pathogenic.

Table 3: Percentage of wilt incidence (%) on chickpea host differentials against different isolates of *Fusarium oxysporum* f.sp. *ciceris*

Isolate name	Place of collection	Wilt incidence (%)									
		JG-62	JG-74	C-104	WR-315	CPS-1	BG-212	Chaffa	KWR-108	DCP-92-3	GPF-2
Foc-1	Yemmiganur	63.33	13.33	40.00	20.00	20.00	16.66	56.66	20.00	60.00	20.00
Foc-3	Kodumur	66.66	10.37	40.00	20.00	20.00	20.00	16.66	20.00	50.00	20.00
Foc-4	Nandyal	80.00	23.33	30.00	16.66	20.00	20.00	70.00	20.00	60.00	12.50
Foc-6	Allagadda	100	26.66	60.00	20.00	20.00	16.66	90.00	23.33	70.00	10.00
Foc-8	Ongole	80.00	13.33	16.66	20.00	10.00	13.33	23.33	06.66	36.66	06.11
Foc-9	Addanki	37.77	10.00	20.00	13.33	20.00	13.33	23.33	20.00	26.66	11.66
Foc-10	Marripudi	100	26.66	30.00	20.00	20.00	20.00	70.00	20.00	70.00	09.40
Foc-12	Cumbum	100	26.66	40.00	20.00	20.00	20.00	70.00	36.66	60.00	20.00
Foc-14	Parigi	60.00	10.00	23.33	20.00	16.66	13.33	60.00	20.00	56.66	16.66
Foc-15	Kalyanadurgam	60.00	06.00	23.33	10.00	16.66	13.33	60.00	23.33	26.66	13.33
Foc-17	PD palli	100	16.66	40.00	16.66	16.66	20.00	50.00	20.00	60.00	04.10
Foc-19	Kaligiri	10.00	00.00	00.00	00.00	00.00	00.00	06.66	00.00	00.00	00.00
Foc-21	Vinjamur	73.33	20.00	20.00	20.00	10.00	16.66	46.66	16.66	20.00	13.33
Foc-22	Tullur	66.66	10.00	33.33	16.66	10.00	20.00	53.33	20.00	30.00	13.33
Foc-23	Tadikonda	40.00	16.66	36.66	20.00	16.66	13.33	66.66	20.00	33.33	09.44
Foc-24	Duttalur	93.33	23.33	26.66	20.00	20.00	20.00	70.00	03.33	60.00	20.00
Foc-26	Vempalli	73.33	20.00	20.00	16.66	10.00	20.00	50.00	20.00	40.00	16.66
Foc-28	Proddutur	40.00	10.00	13.33	06.66	08.88	06.66	30.00	16.66	40.00	16.66
Foc-30	Adilabad	80	23.33	60.00	20.00	16.66	20.00	70.00	20.00	60.00	20.00
Foc-32	Bodhan	100	10.00	40.00	10.00	20.00	13.33	20.00	13.33	40.00	16.66

Resistant: 0 to 20%, Moderately susceptible: 20 to 50%, Highly susceptible: 50 to 100% of wilt incidence.

Table 4: Disease reaction of chickpea host differentials against different isolates of *Fusarium oxysporum* f.sp. *ciceris*.

Isolates	Place of collection	JG-62	JG-74	C-104	WR-315	CPS-1	BG-212	Chaffa	KWR-108	DCP-92-3	GPF-2
Foc-1	Yemmiganur	S	R	MS	R	R	R	S	R	S	R
Foc-3	Kodumur	S	R	MS	R	R	R	R	R	MS	R
Foc-4	Nandyal	S	MS	MS	R	R	R	S	R	S	R
Foc-6	Allagadda	S	MS	S	R	R	R	S	MS	S	R
Foc-8	Ongole	S	R	R	R	R	R	MS	R	MS	R
Foc-9	Addanki	MS	R	R	R	R	R	MS	R	MS	R
Foc-10	Marripudi	S	MS	MS	R	R	R	S	R	S	R
Foc-12	Cumbum	S	MS	MS	R	R	R	S	MS	S	R
Foc-14	Parigi	S	R	MS	R	R	R	S	MS	S	R
Foc-15	Kalyandurgam	S	R	MS	R	R	R	S	MS	MS	R
Foc-17	PD palli	S	R	MS	R	R	R	MS	R	S	R
Foc-19	Kaligiri	R	R	R	R	R	R	R	R	R	R
Foc-21	Vinjamur	S	R	R	R	R	R	MS	R	R	R
Foc-22	Tullur	S	R	MS	R	R	R	S	R	MS	R
Foc-23	Tadikonda	MS	R	MS	R	R	R	S	R	MS	R
Foc-24	Duttalur	S	MS	MS	R	R	R	S	R	S	R
Foc-26	Vempalli	S	R	R	R	R	R	MS	R	MS	R
Foc-28	Proddutur	MS	R	R	R	R	R	MS	R	MS	R
Foc-30	Adilabad	S	MS	S	R	R	R	S	R	S	R
Foc-32	Bodhan	S	R	MS	R	R	R	R	R	MS	R

R: Resistant, MS: Moderately susceptible, S: Susceptible.

Table 5: Sequences (5′ to 3′) of SCAR primers designed.

Primer name	Sequence (5′ to 3′)
SCAR 1F	TACGGCTGGCCGATTAGA
SCAR 1R	TACGGCTGGCGGCAGG
SCAR 2F	CGGCTGGCCGATTAGATACC
SCAR 2R	TACGGCTGGCGGCAGGAC

Chaffa and Annegiri besides CPS-1 and C-104 (moderate susceptible to resistant) were categorized as race-6.

The results are in accordance with Haware and Nene (1982) who reported four races of Foc (race-1, race-2, race-3 and race-4) in India and presented race 1 from Hyderabad. They grouped Foc isolates as race-1 based on disease reaction of two cultivars such as CPS-1 and BG-212. Dubey *et al*. (2012) reported two cultivars C-104 and GPF-2 as differentials (showing resistant reaction) for Foc isolates of A.P and reported race-1 in A.P and Karnataka. In this, susceptible, moderately susceptible and resistant reaction was found with cultivar C-104 for 17 isolates and these isolates were categorized as race-1 based on resistant reaction on CPS-1, BG-212 and GPF-2. Previous studies also reported race-1 from A.P (Haware and Nene, 1982) and Karnataka states (Reddy and Dubey, 2006). In our study also the results confirmed the presence of race-1 in Andhra Pradesh.

Phillips (1988) first observed the presence of race-6 in California depends on resistant reaction on Chaffa, Annegiri and K-850 as it was previously reported as race-1. Reddy and Dubey (2006) reported three new races such as race-5, race-6 and race-7 in India along with previously reported races such as race-1, race-2, race-3 and race-4. They have categorized isolates of Foc into race-1 based on differential reaction of K-850, C-104, WR-315, BG-212, CPS-1 and race-6 based on disease reaction of Annegiri which has given resistant reaction along with WR-315, CPS-1 and C-104. Hence, in this study these two isolates (Foc-3 and 32) were categorized as race-6. Sharma *et al*. (2014) collected 110 isolates from five different zones of India and reported race-1, race-2, race-4, race-6 and new reaction (14 isolates) on differentials such as JG-62, L-550, JG-74, Chaffa, C-104, CPS-1, WR-315, BG-212, Annegiri and K-850. They reported race-1 and race-6 from Andhra Pradesh. In this study, out of 20 isolates studied, 17 isolates showed the similar reaction of race-1, two isolates showed as race-6 and one isolate showed new reaction and this isolate (Foc-19) exhibited 0% incidence on differential lines except JG-62 (10% incidence) and Chaffa (6.66%).

Sharma *et al*. (2014) reported the presence of two races such as race-1 (47%) and race-6 (29%) dominantly out of 110 isolates studied and found that race-1 was widely distributed because of its high genetic diversity and wide inhabitation under the present conditions in all agro ecological zones of India with predominantly in southern zone. It might be due to the large exchange of germplasm and transmission of seeds that took place high in last one decade. In this study also, 17 isolates were grouped as race-1, two Foc isolates were grouped as race-6. Correll, 1991 reported that among all the races, race-1 is reported in India, California and Mediterranean region. Previously race-6 was confined to Mediterranean basin and also reported from California (Phillips, 1988) subsequently reported from Spain

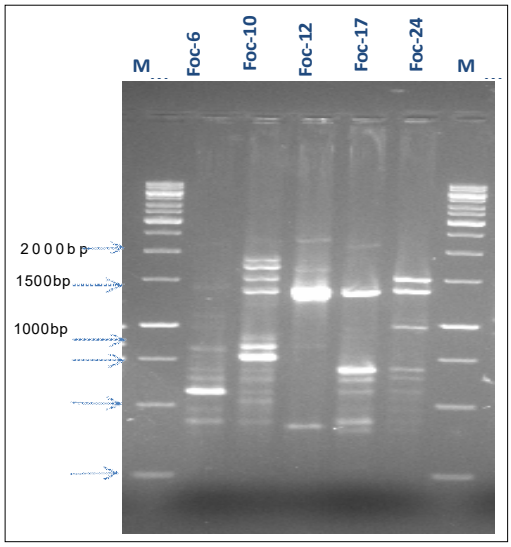

Fig 1: RAPD profiles of five virulent isolates of *Fusarium oxysporum* f.sp. *ciceris* obtained with K5 primer. M: Marker (1 kb).

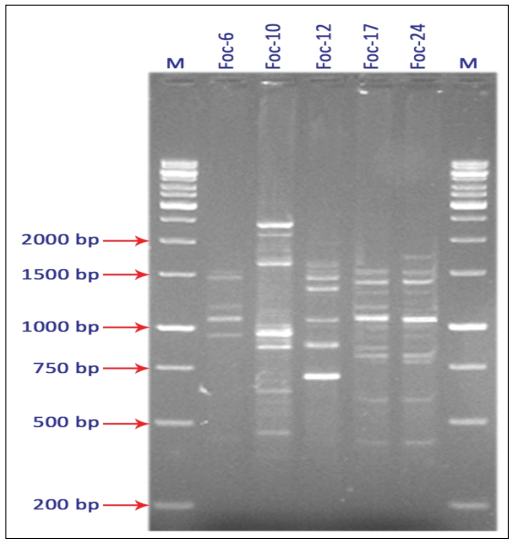

Fig 3: RAPD profiles of five virulent isolates of *Fusarium oxysporum* f.sp. *ciceris* obtained with P2 primer. M: Marker (1 kb).

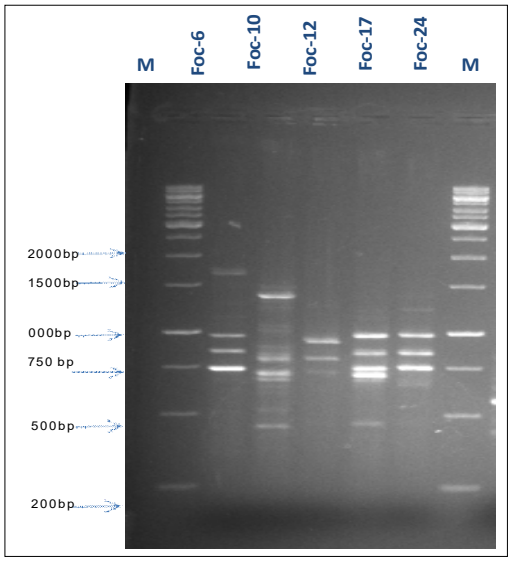

Fig 2: RAPD profiles of five virulent isolates of *Fusarium oxysporum* f.sp. *ciceris* obtained with K6 primer. M: Marker (1 kb).

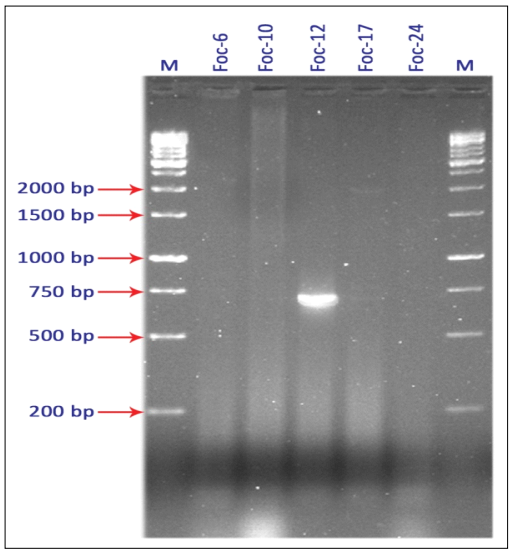

Fig 4: Agarose gel showing amplification product of approximately 700 bp by using SCAR primers M: Marker (1 kb).

(Jimenez-Diaz *et al*. 1989), Israel and Morocco (Kelly *et al*. 1994). Existence of this new race of Foc in India might be a recent introduction through seed transmission by huge interchange of germplasm and accumulation of mutations, which caused genetic variability of pathogen over the period of time.

Stepwise evolution of races in Foc resulted from specific resistance assortment in populations of chickpea or repeated migration might have happened rather than the evolution of races individually in different places or might be expected from an ancient heredity of race-1, it could be the common progenitor of all races (Sharma *et al*., 2014). Additionally, there are reports that races-1 and 6 have similar virulence pattern on host differentials (Halila and Strange, 1996 and Jimenez Diaz *et al*., 1993), having high genetic similarities and shared some RAPD markers (Jimenez-Gasco *et al*., 2001). It was further supported by Jimenez-Gasco and Jimenez-Diaz (2003) who developed RAPD-SCAR common marker for race 1A and race 6 (FocR0-M15r, FocR6-P18f), which produced a single product of nearly 1,400-bp from genomic DNA of *F. oxysporum* f.sp. *ciceris* isolates fitting to races 1 and 6. Former researchers directed that it is also possible that mis categorization of these isolates ensued during pathogenicity test and evolutionarly also these two races were not yet separated from each other and to have developed diagnostic and separate pathogenicity test.

In this study, Foc isolates differed in wilt incidence on host differentials and also showing different pattern of

virulence in some host differentials. Though farmers in Andhra Pradesh and Telangana chosed JG-11 as a major variety for cultivation, other varieties like KAK-2, NBeG-3, NBeG-47, Vihar and JAKI-9218 were also grown in some areas. In A.P, in recent years chickpea cropped area was very much increased than earlier years. Sharma *et al*. (2014) mentioned that dissemination of virulence within and among populations of Foc may be the result of various selection pressures exerted by a specific resistant gene in the varieties of chickpea growing in that area. They also proposed that it could be the result of transfer in the cultivation of chickpea from northern India (Gaur and Gowda, 2005) to southern India (In A.P. itself, chickpea area was 2.85 lakh ha in 2001-02 and raised to 6.02 lakh ha in 2006-07, source: www.indiastat.com) which further supported the low frequency of race-2, which was originally reported from Kanpur, north India and complete absence of race-3 disease reaction in any of the collected isolates. At the same time, irrespective of the origin, the similarities of race composition between the different zones of India indicated repeated migration of infected seeds. In the current study, race-6 reaction might be due to the cultivation of new varieties (pathogen is seed borne), brought from research stations or some other places and also accumulation of mutations over period of time. Hence, it is important to check the susceptibility of widely grown chickpea genotypes to Fusarium wilt, required continuous monitoring of changes in Foc group to know the racial outline.

Development and Validation of SCAR marker

The banding pattern obtained in RAPD with primers K5, K6 and P2 along with 1 kb marker was represented in Fig 1, 2 and 3. The amplified product of polymorphic band of approximately 700 bp in the isolate Foc-12, obtained during RAPD analysis with P2 primer was selected for cloning and SCAR marker development. Two sets of primers *i.e*., SCAR 1 (SCAR 1F/SCAR 1R) and SCAR 2(SCAR 2F/SCAR 2R) were designed (Table 5) based on a terminal sequence of amplified 700 bp DNA from Foc-12. The designed primers were synthesized at Sigma Aldrich chemicals Pvt.Ltd, Bengaluru.

These SCAR markers were validated for the five Foc isolates such as Foc-6, Foc-10, Foc-12, Foc-17 and Foc-24. The results of validation indicated that the same fragment size (700 bp) was obtained in Foc-12 with the primer pair of SCAR 1F/SCAR 2R and SCAR 2F/SCAR 1R and this band was absent in the remaining four isolates such as Foc-6, Foc-10, Foc-17 and Foc-24. Thus this primer set was successfully validated (Fig 4). Thus the certainity of these markers were confirmed, where as with other primer set (SCAR 1F/SCAR 1R and SCAR 2F/SCAR 2R) a thin DNA fragment (700 bp) was noticed in Foc-12, not resembling with other pair, where thick and conspicuous DNA fragment of same size with same molecular weight was obtained. All the five isolates including Foc-12 are showing race-1 disease reaction on host differentials.

Jimenez-Gasco and Jimenez-Diaz (2003) developed SCAR primers by using RAPD markers for Foc isolates and races 0, 1A, 5 and 6. In this study, SCAR marker was developed for Foc-12 isolate but not for race-1. Similarly, Dubey (2010) developed markers based on ITS region for identification of Foc population in India. Durai *et al*. (2012) developed first time two sets of primer pairs SC-FOC1 and SC-FOC2 for identification of Foc population and they were checked against 36 collected Foc isolates representing different races present in India and confirmed the specificity of those markers. Katkar *et al*. (2015) validated the specific SCAR primers for FOC (developed by Jimenez-Gasco and Jimenez-Diaz, 2003) and for all four Indian races such as race-1, 2, 3 and 4.

In the existing study, diverse size of bands were observed in all the five belonging to same race (race-1) during RAPD analysis and the pattern of bands was not capable to distinguish them. This might be due to random nature of RAPD markers and seemed to be unable to focus the genetic regions that are responsible for specification of races. Understanding the genetic mechanisms of race cultivar specificity in *F. oxysporum* are largely unknown, due to the absence of a sexual stage in this fungus and genetic analysis (Huertas-Gonzalez *et al*., 1999) is difficult. Thus, the selected unique band in Foc-12 was validated with the developed SCAR markers.

CONCLUSION

Out of 20 Foc isolates collected from A.P and Telangana, 17 of them were matched with race-1 reaction, two isolates matched with race-6 reaction. SCAR markers developed and successfully validated for isolate Foc-12 out of five isolates. Races identification helps to chickpea breeders to develop race specific resistant cultivars in different areas and also framing of good management practices for Fusarium wilt in Andhra Pradesh and parts of Telangana.

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