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Effect of temperature on the severity of *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris*

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Abstract
Under changing climatic scenario, the rise in temperature has become a major issue, particularly in the semi-arid tropics. As a result of it, a drastic shift in chickpea diseases has been recorded throughout the major chickpea growing regions of India. The *Fusarium* wilt of chickpea caused by *Fusarium oxysporum* f. sp. *ciceris* (*Foc*) is one of the major potential constraint for chickpea production. The present study, therefore, was focused on assessing the impact of different levels of temperature on *Foc* virulence in different chickpea cultivars. The *Foc* incidence was significantly affected by higher temperature. Out of six temperature levels (15, 20, 25 30, 35 and 40 °C), higher temperature levels of 25, 30 and 35 °C, predisposes chickpea to *Foc* infection with cent per cent disease incidence. The study indicates that temperature plays a vital role in maintaining the virulence of *Foc* and predisposes chickpea to *Fusarium* infection and disease development.

Keywords: Chickpea, *Foc*, climate change and temperature

Introduction
Chickpea (*Cicer arietinum*. L) is an annual cool-season legume crop and is grown in several countries worldwide as a food source. Chickpea is originated from south-eastern Turkey, from where it has spread to other countries of the world (Ladizinsky, 1975) [1]. It is the largest produced food legume in South Asia and the third-largest globally, after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.). In India, chickpea holds second in area and third in production, and it is the major producer of chickpea accounting for 75 per cent of world production. In India, a drastic shift in chickpea cultivation from north to the central and southern parts has been reported due to variation in climatic conditions and as a result, a shift in the disease pattern has been found (Gowda and Gaur, 2004) [2]. The vulnerability of chickpea to many biotic and abiotic stresses is a major constraint for reduced yields. Among several diseases of chickpea, wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is the most devastating disease in India. It is typical vascular wilt causing xylem browning or blackening. The wilted plant shows marked discoloration of the vascular tissues in the stem (Haware *et al.* 1982) [3]. With reference to crop losses, rough estimates indicated that *Fusarium* wilt causes losses around 10 to15 per cent each year as a regular feature.

Host and pathogens are influenced by the interactive effects of multiple climatic factors (Chakraborthy, 2013) [4]. It is just one of the ways in which the environment can move in the long term from disease-suppressive to disease-conducive or vice versa (Chakraborty and Datta, 2003) [5]. The variations in the climatic factors such as an increase in CO_2 concentrations, unanticipated rainfall patterns, temperature rise are mainly influencing the plant pathogen establishment (Graham and Vance, 2003) [6] and also affects the virulence of many pathogens (Coakely *et al.* 1999) [7].

Very little information is known about the epidemiology of the disease in relation to the predisposition of chickpea plants by different levels of temperature, as mainly high temperature promotes infection and development. So the present study was conducted to know the effect of different level of temperature on the virulence of *Foc*, infection and disease expression under controlled environment. The study was initiated to know the most favorable temperature for growth of *Fusarium* in different chickpea cultivars.

Materials and Method

Virulence analysis of *Foc* isolates using standard differentials

The three *Foc* isolates (*Foc* 311, *Foc* 312 and *Foc* 314) were used for host differentials study based on the pathogenicity, cultural and morphological characters which were collected from different fields of ICRISAT. In the present investigation, ten standard chickpea host differentials *viz*., JG 62, C104, WR 315, Annigeri, Chafa, K 850, L 550, CPS 1, BG 212 and JG 74 were used, which were collected from the Legume Pathology Division, ICRISAT, Patancheru. Raising of seedlings, inoculum preparation, inoculation, transplanting of seedlings and disease reaction were recorded as per the procedure described by Pande *et al.* (2012) [8].The plants were kept in the greenhouse condition at a temperature of 25 ± 2 ºC with 12 h natural light per day. Wilt incidence was recorded periodically at two days intervals starting from ten days after transplanting and final observations were recorded after 30 days of transplanting. The disease incidence was calculated by using the following formula

$$\text{Wilt incidence (\%)} = \frac{\text{Number of plants wilted}}{\text{Total number of plants}} \text{ X } 100$$

Table 1: Disease reaction category based on disease incidence

Sl. No.	Disease incidence	Disease reaction	Virulence level
1.	0-10%	Resistant	Least virulent
2.	10.1-20.0%	Moderately resistant	Moderately virulent
3.	20.1-40.0%	Moderately susceptible	Virulent
4.	40.1-100%	Susceptible	Highly virulent

Highly virulent isolate was selected based on the incubation period and disease reaction of three isolates on differentials.

Morphological characters of virulent isolate under different temperature levels

To know the most suitable temperature for mycelial growth and sporulation of *Foc* the experiment was conducted in incubators. The sterilized poured Petri plates with PDA were inoculated with 5 mm disc of the test pathogen of seven days old culture. The Petri plates were incubated at 10, 15, 20, 25, 30, 35, and 40 ºC temperature. For each treatment three replications were maintained and observation for mycelial growth and dry mycelial weight was recorded after seven days. Sporulation was recorded at seven days after inoculation with the help of hemocytometer.

Virulence of the *Foc* on resistant and susceptible cultivars of chickpea under a range of temperature

Wilt susceptible (JG 62 and C104) and resistant (WR 315 and JG11) cultivars of chickpea and highly virulent isolate (*Foc* 314) were selected based on the disease reaction of three *Foc* isolates on ten differentials. Six temperature regimes (15 ºC, 20 ºC, 25 ºC, 30 ºC, 35 ºC and 40 ºC) were selected for virulence analysis of *Foc* on chickpea cultivars. Raising of seedlings, inoculum preparation, inoculation and transplanting of seedlings were done as per the procedure described by Pande *et al.* (2012) [8]. After transplanting in 5 inch plastic pots filled with sterilized soil media, the pots were then transferred to the incubators adjusted with different temperature levels of 15, 20, 25, 30, 35 and 40 ºC. Three replicate pots of each treatment were arranged in a completely randomized design and uninoculated control was kept, where root tips were dipped in sterile distilled water and transplanted into the pots. Incubation period and disease development were assessed by recording disease incidence at every week intervals up to 30 days after shifting into incubator.

Results and Discussion

Virulence analysis of *Foc* isolates

Irrespective of isolates, among ten host differentials, four differentials *viz*., BG 212, CPS 1, JG 74 and WR 315 showed resistant reaction with zero disease incidence. Among three isolates *Foc* 314 isolate showed susceptible reactions in remaining six differentials with variation in virulence level upto 40-100 per cent, such of host differentials include Annigeri, C 104, Chafa, JG 62, K 850 and L 550. However, *Foc* 311 and *Foc* 312 isolates showed susceptible reaction in only two differentials such as JG 62 and C 104 followed by moderately susceptible in Chafa. Based on the reactions of differentials, among three isolates, *Foc* 314 isolate is considered as highly virulent isolate and two susceptible (JG 62 and C 104) and resistant cultivars (WR 315 and JG 11) were selected for further studies (Fig. 1).

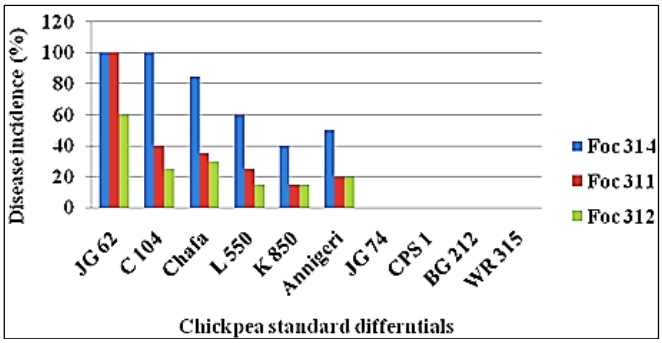

Fig 1: Reaction of *F. oxysporum* f. sp. *ciceris* isolates on chickpea differentials for virulence profiling using rapid root dip inoculation

Morphological variability of *Foc* 314 isolate at different temperature

Morphological features of *Foc* 314 isolate of *Foc* were studied by growing at different temperature *viz.*, 15, 20, 25, 30, 35 and 40 ºC on the PDA media. It is characterized with respect to different parameters such as mycelial growth, number of spores per ml and dry mycelial weight to assess the existence of variation at different temperature. It was seen that there was quite a large variation in the growth of *Foc* 314 isolate at different temperature. The highest growth of the pathogen was recorded at 30 ºC (89.40 mm) followed by 25 ºC where it attained the maximum growth 87.32 mm while at 20 ºC and 35 ºC, it was 41.08 and 51.21 mm which showed significantly lesser growth than 30 ºC and no growth of the pathogen was observed at 40 ºC.

Observations on oven dry mycelial revealed that, highest mycelial dry weight was observed in 25 and 30 ºC with 167.21 and 166.24 mg followed by 35 ºC were 85.34 mg were recorded which, differed significantly at different temperature levels. Very less mycelia dry weight was observed in 15 and 20 ºC. However, similar trend was observed in sporulation also, were maximum sporulation of 2.17×10^6 spores/ml and 2.19×10^6 spores/ml was produced in 25 and 30 ºC followed by 35 ºC whereas least sporulation was produced in 15 ºC (Table 2).

Table 2: Morphological diversity of *Foc* 314 isolate of *F. o* f.sp *ciceris* of chickpea

Sl. No	Temperature (°C)	Mycelial growth (mm)				Conidia Number (10^6/ml)	Dry mycelia weight (mg)
		2 DAI	4 DAI	6 DAI	8 DAI		
1	15	16.66	20.36	23.33	23.38	0.13	37.12
2	20	18.33	32.34	41.02	41.08	0.47	45.21
3	25	32.62	44.67	70.83	87.32	2.17	167.21
4	30	33.33	46.35	72.35	89.40	2.19	166.24
5	35	21.34	3.60	40.82	41.21	1.98	85.34
6	40	0	0	0	0	0	0
S.Em ±		0.34	0.92	0.52	0.82	0.15	0.06
CD @ 0.01%		0.91	2.93	1.72	2.78	0.47	0.18

3.3 Virulence of *Foc* 314 isolate on resistant and susceptible cultivars under a range of temperature

All plants grown in non-inoculated pots (Controls) remained healthy at all temperature regimes throughout the experiment. Irrespective of cultivars no disease incidence was recorded when inoculated plants were maintained at 15 °C. Delayed disease progression was noticed with incubation period (IP) of 22.64 days, in both JG 62 and C 104 cultivars when inoculated plants were maintained at cooler temperature of 20 °C.

Disease was more severe under warmer conditions, in both JG 62 and C 104 cultivars at 25 and 30 °C, where initial symptoms started with IP of 11.34 and 11.14 days and 100 per cent disease incidence (DI) was noticed with typical wilt symptoms which differed significantly from other temperature levels. At 35 °C, in both JG 62 and C 104 cultivar incubation period was delayed by 2 to 3 days when compared to 25 and 30 °C and 100 per cent disease incidence was noticed at 28 DAI. No disease incidence was recorded in WR 315 and JG 11 cultivar irrespective of temperature regimes. It was observed that 40 °C was not suitable for normal growth of plants, because physiological wilting of plants were noticed in all four cultivars and also this extreme temperature was not favourable for growth of *Foc* (Fig. 2).

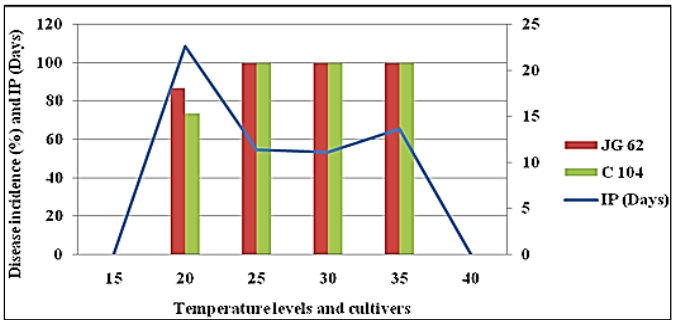

Fig 2: Effect of temperature on incidence of *Fusarium* wilt of chickpea in different cultivars

Discussion

Virulence is defined as a quantitative measure of pathogenicity denoting the severity of disease caused by a pathogen on a particular host (Parker and Gilbert, 2004) [9]. This is mainly affected by the abiotic factor like temperature, which can significantly influence the development of *Fusarium* wilt of chickpea (Cortes *et al*. 2000) [10]. The results of the virulence analysis of *Foc* isolates on chickpea differentials indicated that highly virulent isolate (*Foc* 314) induced 100 per cent wilting on JG 62 with the early incubation period. The results of the present findings were in conformity with the findings of Manish *et al.* (2015) [11], wherein they categorized *Foc* isolates as highly virulent those inducing 100 per cent wilting within 25 days of sowing. Similar variations in the virulence assay of 41 isolates of *Foc* was earlier observed by Sharma *et al*. (2009) [12]. They recorded 8 to 20 days of incubation period in case of chickpea. Similar categorization of *Fusarium* isolates based on the pathogenic reaction was noticed on JG-62 cultivar of chickpea, which was earlier carried out by Trivedi and Chaudhary (2011) [13].

In the Indian climatic condition, chickpea is grown during the post-rainy season on receding soil moisture, leading to terminal drought stress. In the present study, optimum temperature observed for the growth of *Foc* on PDA was 25 and 30 ˚C, however very less growth was observed on 15 and 20 ˚C. Few earlier studies indicated that *Foc* showed vigorous growth at 30 °C while growth was less vigorous at 40 °C (Khan *et al.* 2012) [14]. Morphological characters like colony growth pattern, size of colony and pigmentation, a number of conidia were also influenced by variation in temperature level, these results were well supported by observation made by other scientists also (Dubey *et al.* 2012 [15]; Mandhare *et al.* 2011) [16].

In the present study we noticed that temperature plays a major role in disease development. We observed that on JG 62, 100 per cent disease incidence was recorded at 25 and 30 °C with early incubation period of 12-13 days however, at low temperature delayed incubation period with less disease incidence was observed. The high temperature of 40 °C is detrimental to the plant growth regardless of whether the pathogen was present or not, and this can be attributed to the significant effect of temperature on plant growth and development. In particular, the temperature may modify plant-pathogen interactions by affecting metabolic processes and development of the plants, as well as pathogen growth and virulence. Understanding the precise influence of temperature on *Fusarium* wilt of chickpea is essential for management of the disease. Several studies reported the critical role of temperature on the development of *Fusarium* wilt of chickpea. Earlier reports indicate that it is generally favored at a temperature range of 20 to 30 °C (Landa *et al.* 2001) [17], with the optimum temperature for disease development between 24.5 and 28.5 °C.

The disease incidence and incubation period were directly proportional to each other. Apparent infections were seen 11-12 days after the inoculation in 25 and 30 °C, indicating that temperature is a predisposing factor for initial infection of host tissue. Similarly, Scott *et al.* (2010) [18]. Observed the effect of temperature on *Fusarium* wilt of lettuce (*Lactuca sativa*) and disease was observed to increase from 10 °C up to an apparent maximum near 25 °C.

This indicates that the pathogen was sensitive to very high and low temperature and cannot survive for a longer period under very less temperature. Consequently, the lack of consideration of the effects of temperature on *Fusarium* wilt may lead to the wrong race and virulence characterization of isolates of *Foc*. Harling *et al.* (1998) [19]. Reported that *F. oxysporum* f. sp. *dianthi* remained symptomless at 14 to 15 °C, but the cultivars could be differentiated into resistant, partially resistant and susceptible types at 22 °C.

Conclusion

The study clearly demonstrates that temperature has become a major climatic factor influencing the *Foc* infection, incubation period and disease development in chickpea. This study will substantially accelerate the on-going efforts to understand the disease triangle interactions in chickpea under the changing

scenario of climate. Also, a better understanding of the role of temperature will help in standardization of *Foc* resistance screening techniques and will assist breeders in optimization breeding strategy for *Foc* that will enable long-term resistance over broader geographical areas.

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