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Establishment of the Chickpea Wilt Pathogen *Fusarium oxysporum* f. sp. *ciceris* in the Soil through Seed Transmission

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Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (FOC) is the most destructive disease in India. It is seed-borne as well as soil-borne pathogen. The role of seed-borne FOC in introducing and establishing wilt in FOC free soils is unknown. Using seeds of FOC infected chickpea cultivar K 850, we provided an evidence of establishing wilt disease in the FOC free soils within three crop cycles or seasons. In the first cycle, typical wilt symptoms were observed in 24 pots in 41 days after sowing. These 24 pots were used for second and third cycles without changing the soil. These 24 pots were sown with seeds collected from healthy plants of a susceptible cultivar JG 62, one seed per pot and development of wilt symptom was recorded. Wilt symptoms appeared in all the pots 26 days after sowing in second cycle and in 16 days after sowing in third cycle. On selective medium, all of the wilted plants yielded FOC in all the three cycles indicating that the mortality was due to wilt. FOC propagules on selective medium were 172, 1197, and 2280 g^{-1} soil at the end of the first, second, and third cycles, respectively. These studies indicated that Fusarium wilt of chickpea is seed-borne and seeds harvested from wilted plants when mixed with healthy seeds can carry the wilt fungus to new areas and can establish the disease in the soil to economic threshold levels within three seasons.

Keywords **:** chickpea, wilt, *Fusarium oxysporum* f. sp. *ciceris*, soil borne, seed borne

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* (FOC) is the most important disease of chickpea (*Cicer arietinum*). It is more prevalent in lower latitudes (0-30°N) where growing season is relatively dryer and warmer than in the higher latitudes (30-40°N). It causes complete loss in grain yield if the disease occurs in the vegetative and reproductive stages of the crop (Haware and Nene, 1980; Haware et al., 1990; Halila and Strange, 1996; Navas et al., 2000). The disease manifests as mortality of young seedlings (within 25 to 30 days after sowing) to wilting or death of adult plants. The fungus FOC is a primarily soil-borne pathogen, however, few reports indicated that it can be transmitted through seeds (Haware et al., 1978).

Seedlings that die due to wilt disease can be confused with other diseases of wilt complex, if not examined carefully. Fusarium wilt infected seedlings collapse and lie flat on the ground retaining their dull green color. Adult plants show typical wilt symptoms of drooping of petioles, rachis and leaflets. The roots of the wilting plants do not show any external rotting but when split open vertically, dark brown discoloration of internal xylem is seen (Nene et al., 1991). Pods from the wilted plants look normal but seeds are generally smaller, wrinkled and discolored. Though such seeds can be detected visually, a normal looking seed harvested from wilted plants may also harbor the wilt pathogen. Seeds from wilted plants when mixed with the seeds from healthy plants may be responsible in introducing wilt in new areas. If FOC inoculum establishes in the soil, it is difficult to check the disease or eliminate the pathogen except by following crop rotation for more than six years (Haware and Nene, 1982; Gupta, 1991). In recent years, incidence of wilt in the farmers' fields is increasing considerably every year and its severity is directly related to the increasing density of the pathogen inoculum in the soil (Bhatti and Kraft, 1992; Sugha et al., 1994; Zote et al., 1996). There is a possibility that increasing density of FOC in the soil to a greater extent is also contributed by the FOC infected seeds mixed with healthy seeds used for sowing purposes. Therefore, the objective of this study was to quantify the progress in establishing FOC in new and healthy soils through chickpea grains harvested from wilted plants and used as seeds to raise a chickpea crop.

Materials and Methods

Collection of seeds. Seeds were collected from wilted plants of the chickpea cultivar K 850 grown in wilt sick plot at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India during 2004-2005 crop season. These seeds were air dried at room temperature and stored at 5°C in the refrigerator in the legumes pathology laboratory at ICRISAT.

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Quantification of FOC in the seeds collected from wilted plants. Following standard procedures of seed health testing (Haware et al., 1978), intensity of the wilt fungus in the seeds of chickpea cultivar K850 collected from wilted plants was quantified. Seeds of K 850 collected from healthy plants were used as control. The experiment consisted of four replications with 100 seeds in each replication and repeated once. Thus 400 seeds each collected from wilted and healthy plants were surface sterilized with 2.5% Clorox for 5 min and were plated onto the modified czapek dox agar medium in 10 cm glass petri plates, 10 seeds per plate. All the plates were incubated in Percival incubator at 25ºC with 12 h light and 12 h dark periods. Data on number of seeds colonized with FOC were recorded at seven days after incubation.

Pathogenicity of the *Fusarium* isolates. All of the *Fusarium* isolates that colonized the wilted seeds of the cultivar K 850 were separately multiplied on sterilized potato dextrose broth in 250 ml conical flask and incubated for 7 days at 25ºC and 125 rpm. This culture was then homogenized in sterile distilled water and adjusted to 5×10^5 conidia ml^{-1} and used as inoculum. Eight-day-old seedlings of a susceptible cultivar JG 62, grown in sterile sand were uprooted, sand particles were removed from the roots by washing with distilled water, and root-inoculated by dipping in the inoculum for 30 seconds to enable conidia to adhere to the roots. Root-inoculated seedlings were transplanted in pre-irrigated sterile vertisol and sand (3:1) pot mix filled in 15 cm plastic pots and kept in a greenhouse at 25±3ºC. Inoculated seedlings were observed for wilt symptoms up to 30 days after inoculation.

Establishment of FOC in the soil through infested seed. To quantify the number of cycles (crop seasons) required for the establishment of FOC in the FOC-free soil through infested seeds, pot experiments were conducted in the greenhouse at ICRISAT. Chickpea was sown in three successive cycles representing three crop seasons in the same pots without changing the soil. The soil used in this experiment was sandy-loam and was sterilized for one hour at 121ºC and 20 lb pressure. About 500 g of the sterilized soil filled in 10 cm diameter plastic pots was used for sowing chickpea seeds. Seedlings were observed up to 45 days after emergence for wilt disease symptoms in each cycle. Temperature of the greenhouse was maintained at 25±3ºC during the experimentation period. The experiment was repeated once. Schematic diagram representing the establishment of FOC in the new soil through seeds is presented in Fig. 1.

First cycle representing the first crop season. One hundred seeds each collected from wilted and healthy plants of the cultivar K850, were surface-sterilized and were sown one seed in one pot. Pots sown with seeds obtained from healthy plants served as control. Isolations were made from wilted plants on selective medium for confirmation of the disease. At the end of the experiment *i.e.* 45 days after sowing, 5 g of soil from each of the pots that had produced wilt was taken, thoroughly mixed, processed and assessed for FOC propagules on selective medium (Singh and Chaube, 1970). Roots of the wilted plants were cut into pieces and incorporated in the soil of the same pot while stirring the pots with sterilized iron rod. The pots were given light irrigation to make the soil moist and were undisturbed for a week. These pots were used for second

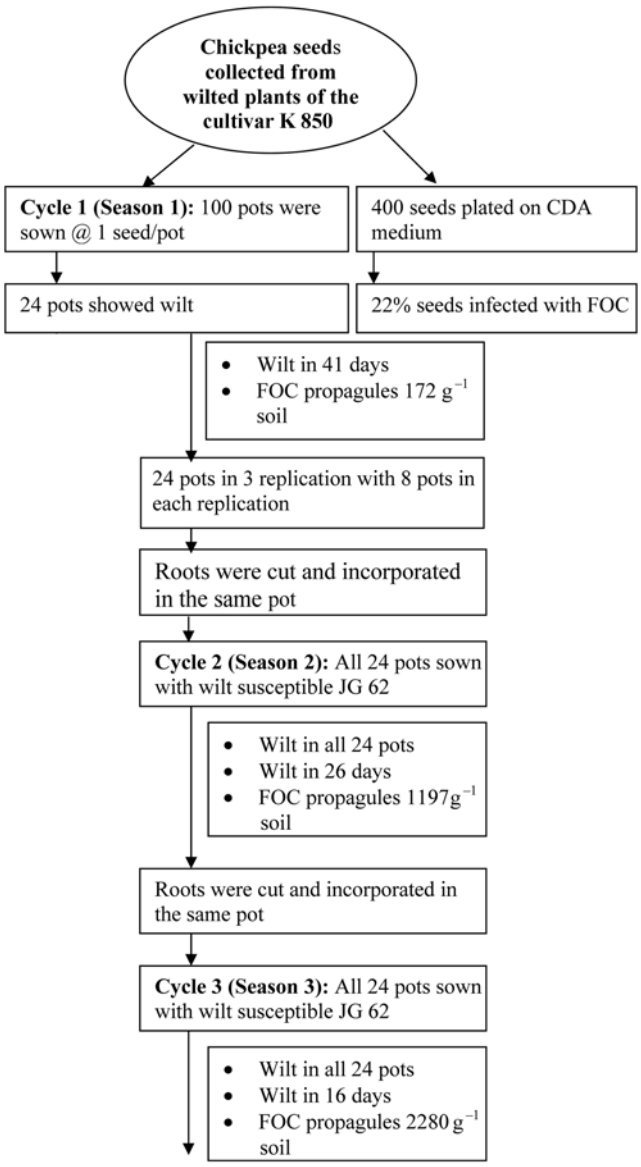

Fig. 1. Schematic diagram showing establishment of *Fusarium oxysporum* f. sp. *ciceris* in soil through infected seeds.

cycle.

Second cycle representing second crop season. Only those pots that had produced wilt in the first sowing were arranged in three replications and used for second and third cycles. The pots that had healthy plants were not used for further experimentation and were discarded. Since late wilting cultivar K 850 can not exhibit wilt symptoms in 45 days after sowing under low FOC population, seeds collected from healthy plants of the wilt susceptible cultivar JG 62 were surface-sterilized and were sown one seed in one pot. Control pots filled with sterilized soil were sown with healthy seeds of JG 62 one seed in one pot. The plants were observed for 45 days for the development of wilt disease symptoms. Wilted plants were collected and isolations were made on selective medium for confirmation of wilt disease. At the end of the second cycle small quantity of soil was collected from all the pots, mixed thoroughly, processed and were assessed for FOC propagules as in the first cycle. Roots of the individual plants were cut into small pieces and incorporated in the soil of the same pot as in the first cycle. Pots were given light irrigation and were undisturbed for seven days before using for third cycle.

Third cycle representing third crop season. In the third cycle also, seeds collected from healthy plants of the wilt susceptible cultivar JG 62 were surface-sterilized in and were sown one seed/pot in all the pots that had shown wilt consecutively in the second cycle (second season). Control pots were sown with healthy seeds of the wilt susceptible cultivar JG 62 as in the second cycle. Plants were observed for wilt disease symptoms for 45 days after sowing. Wilted plants were isolated on selective medium to identify the causal organism for mortality. As in the other cycles soil samples were collected from the pots that had produced wilt and assayed for FOC propagules.

Data analysis. Percentage of FOC colonization on seeds collected from wilted plants, percentage of wilt, number of days to wilt and FOC propagules g/soil were similar in both tests and were found insignificant between tests. Hence, the mean data of both tests was analyzed using analysis of variance (ANOVA). Least significant difference (LSD) at 5% level of significance was used to assess significance of difference of means among different cycles.

Results

Quantification of FOC in the seeds collected from wilted plants. About 22% of the seeds collected from wilted plants of the cultivar K 850 were colonized with FOC on selective medium. No FOC was observed from the seeds collected from healthy plants of the same cultivar (Table 1). These results indicated that ≥20% of the seeds harvested from wilted plants carry FOC.

Table 1. Quantification of *Fusarium oxysporum* f. sp. *ciceris* (FOC) from seeds collected from wilted plants of the cultivar K 850

Source of the seed	Number of seeds plated	Percent colonization with FOC
Wilted seeds[a]	400	22.0
Healthy seeds[b]	400	0.0

[a]Collected from wilted plants of the cultivar K 850
[b]Collected from healthy plants of the cultivar K 850

Table 2. Days to wilt after sowing and number of propagules of FOC from soil collected from the pots where wilt was observed

Cycle	No. of days to wilt	FOC propagules (g^{-1} soil)
1st Cycle[a]	41	172
2nd Cycle[b]	26	1197
3rd Cycle[c]	16	2280
LSD @ 5%	5.3	379.1
CV%	11.5	18.0

[a]Represents virgin soil where chickpea is grown for the first time. Data were based on 100 seeds collected from wilt infected cultivar K 850.
[b]Represents second crop season where chickpea is grown for the second consecutive year. Data was based on 24 pots.
[c]Represents third crop season where chickpea is grown for the third consecutive year. Data was based on 24 pots.

Pathogenicity of the *Fusarium* isolates. Wilt susceptible cultivar JG 62 exhibited wilt symptoms between 12 to 15 days after inoculation in all the Fusarium isolates that colonized the wilted seeds of the cultivar K850. Finally 100% wilt incidence was observed at 30 days after inoculation in all the isolates.

Establishment of FOC in the soil through infested seed. Significant differences were observed among days to wilt and FOC propagules g^{-1} soil between the cycles (Table 2).

First cycle representing the first crop season. Of the 100 pots sown, 24 had produced typical wilted plants in 41 DAS while the rest 76 pots had healthy plants and they remained healthy through out the experimentation. Control pots sown with healthy seeds produced healthy plants. FOC was observed on isolation on selective medium from wilted plants indicating that the wilt fungus was the main cause of mortality in these plants. FOC propagules in the pots showing wilted plants were 172 g^{-1} soil at the end of the experiment (Table 2). Soil sampled and bio-assayed from pots that had no wilt and from control pots did not yield any

FOC propagules.

Second cycle representing the second crop season. In the second cycle, all of the pots in all the replications exhibited typical wilt disease symptoms within 26 DAS. None of the control pots showed any wilt disease symptoms. Isolation of the wilted plants on selective medium yielded FOC in all these plants as in the first cycle. Number of propagules of FOC across the pots was 1197 g^{-1} soil (Table 2).

Third cycle representing the third crop season. Typical wilt symptoms were recorded 16 days after sowing in all the pots that had produced disease in the second cycle. Wilt disease appeared substantially earlier compared to first and second cycles. All of the wilted plants yielded FOC on isolation on selective medium as in the earlier cycles. FOC propagules across the pots was 2280 g^{-1} of soil (Table 2).

Discussion

In this study, 22% of the seeds collected from late wilted plants of the cultivar K 850 yielded FOC on selective medium in the laboratory test. Similar level of wilt incidence (24%) was recorded in the pots sown with seeds collected from wilted plants of the same cultivar in the greenhouse experiments. These results provided the experimental evidence on the seedborne nature of FOC and confirm the earlier observations made by Haware et al. (1978).

In general, wilt in the susceptible cultivar JG 62 manifests within three weeks after sowing in the field where FOC propagules are present in abundance. In the present study wilt was observed in 41 days in the first cycle, 26 days in the second cycle and 16 days in the third cycle. The delay in wilting in the first cycle may be because of the fungus colonized in the form of chlamydospores in the seed, which has to germinate and infect the plant. Moreover, the number of FOC propagules was too low (172 g^{-1} soil) to manifest wilt disease and kill the chickpea plant within three weeks, as it happens in the fields repeatedly sown with chickpea. Previous studies reported that at low level of inoculum densities, appearance of wilt disease was delayed in chickpea (Bhatti and Kraft, 1992; Sugha et al., 1994; Zote et al., 1996). As the inoculum densities increases, numbers of days to exhibit wilt disease symptoms decreases in a susceptible cultivar like JG 62. These results are in agreement with the results obtained in the present study as the number of FOC propagules increased by seven folds resulting in wilt appearance within 26 DAS in second cycle. As the FOC propagules population increased by 13 folds in the third cycle in comparison to cycle 1, wilting of JG 62 occurred within 16 days. This was at par with the time taken for wilt appearance at seedling stage in the heavily FOC infested soils such as chickpea wilt sick plots. These studies provided the evidence that Fusarium wilt is seed borne and suggested that apparently healthy-looking seeds from wilted chickpea plants carry the wilt fungus to new locations and can establish the disease in the FOC free soils within three seasons.

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