Proc. Indian natn. Sci. Acad., B 45, No. 4, pp 383-386 (1979)

Pathogenic and Cultural Variation of the Safflower Wilt-Organism *Fusarium oxysporum* f. sp *carthami*

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(Received 16 October 1978; after revision 27 November 1978)

Five isolates of *F. oxysporum* f. sp. *carthami* were obtained from different areas of Varanasi region. Their pathogenic, morphological and cultural characters were found to vary. On the basis of similarities of important characters isolates of *F. oxysporum* f. sp. *carthami* were divided into three groups of physiologic variants. Group 1 comprises of isolates F.O.1 and F.O.5. The second group includes the isolate F.O.2 while isolates F.O.3 and F.O.4 come under the third group.

Introduction

In recent years the wilt disease caused by *Fusarium oxysporum* Sheld. f. sp. *carthami* Klis. and Hous. has appeared as a serious threat to safflower cultivation as it causes heavy damage to the crop, sometimes as high as 25% (Singh et al. 1975). During a survey of the disease in Varanasi region, the plants were noticed to differ markedly in disease expression even in the same field (Chakrabarti & Basuchaudhary 1978). Klisiewicz and Thomas (1970) also observed safflower materials to vary in their disease reaction. On the basis of virulence of the isolates they established two pathogenic races of *F. oxysporum* f. sp. *carthami*. Presence of the pathogenic races might be the contributing factor to the failure in raising resistant safflower variety attempted by Knowles et al. (1968). From the above findings it is well indicated that *F. oxysporum* f. sp. *carthami* may have its pathogenic races in India also. The present paper is an attempt in this direction.

Materials and Methods

Five isolates of *F. oxysporum* f. sp. *carthami* F.O.1 (IMI 186541), F.O.2 (IMI 204053), F.O.3 (IMI 204056), F.O.4 (IMI 186543), F.O.5 (IMI 186539) were obtained from infected roots from different fields on PDA by usual method. Axenic culture of each was obtained by single spore isolation technique and maintained on PDA. Further, safflower stems were inoculated with these axenic cultures and stored in sterile culture tubes at 0(± 2)°C. Fresh cultures were initiated from these materials.

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Comparative study on the symptoms produced by different isolates

Detailed symptomatology was studied on a susceptible cultivar Hungund under glass-house condition. Steam-sterilized soil was inoculated with the three weeks old culture of the pathogen on corn meal-sand medium (5% by weight). Seven days after infestation, five surface-sterilized (with aqueous solution of $HgCl_2$) seeds were sown in each pot and irrigated with tap water. Three pots for each isolate with equal number of uninfested pots (control) were maintained.

Morphological studies of the isolates

(a) Studies on the fungal spores

Isolates of the pathogen were cultured on PDA and incubated at 21°C for seven days when observations on spore size and shape were made. One Huhdred spores of each isolate were observed.

(b) Effect of media on colony characters

Different isolates of the pathogen were cultured on 50ml each of Martin, Czapeck-dox, Richard's and Potato-dextrose broth by inoculating each medium with 1 ml spore suspension (50×10^2 spores/ml) of each isolate and incubated at 21°C for 7 days.

(c) Effect of temperature on growth

Sterilized PDA plates were inoculated in the centre with each isolate and incubated at 21, 26 and 32°C for 7 days. Radial growth was measured, growth rate per day was calculated and type of colony growth was noted.

In all the experiments six replicates of each treatment were maintained.

Results

Comparative study on the symptoms produced by different isolates

Effects of individual isolates on seed germination, development of symptoms and flowering are presented below.

F.O.1: 70% seeds failed to germinate. The disease manifested itself in form of narrow, distorted cotyledonary leaves on all the seedlings. The first leaf was spiral, plants were sick and growth was stunted. Almost all the leaves suffered from severe chlorosis and epinasty. At the sixteenth week after germination the plants recovered some vitality which was evident by the renewal of growth and greenness of the leaves. The diseased plants could not produce flower buds.

F.O.2: Seed germination was not inhibited. Mild infection of the fungus in form of brown dots on the cotyledonary leaves of 10% seedlings were observed. The first leaf was smaller in size. General growth and vigour of the plants were mildly affected. At one stage of the growth chlorosis developed on lower leaves of all the plants, but most of these chlorotic leaves regained their greenness soon while leaves of about 10% plants could not recover and underwent wilting. Flowering appeared to be normal.

F.O.3: 20% seeds did not germinate. 75% of the seedlings were mildly affected; minute brown dots in form of ring appeared on upper surface of the cotyledonary leaves. The first leaf bent inwards. Growth was apparently normal. Chlorosis was conspicuous in all the plants. Later chlorosis was corrected at the time of blossoming. But in some plants the lower leaves could not recover and underwent wilting. Flower formation was not affected.

F.O.4: 30% seeds did not germinate. Cotyledonary leaves of 56% seedlings were twisted, crinkled and with brown dots either scattered on the surface or in form of small ring. The first leaf was narrow, curved, twisted. Growth was stunted. Leaves suffered from chlorosis and necrosis. Flowering occurred.

F.O.5: 30% seed germination was inhibited. Cotyledonary leaves of all the seedlings were rolled upwards. First leaf suffered from chlorosis and epinasty. Growth was stunted. Chlorosis affected all the leaves severely; the leaves turned golden yellow. However, the upper leaves showed partial recovery while the lower ones succumbed to wilting. Flower buds developed partially.

Morphological studies of the isolates

(a) *Studies on the fungal spores*

In F.O.1 and F.O.5 number of macroconidia were abundant while microconidia were few. Macroconidia of F.O.1 and F.O.5 were multiseptate with bold septa, hooked apex and pedicellate base, sporodochia were present, microconidia were oval in shape. In isolates F.O.2 and F.O.3 microconidia were more in numbers, oval or straight, septa of macroconidia were thin-walled. Chlamydospores of F.O.1 and F.O.5 were few and formed after prolonged incubation. In F.O.2 and F.O.3 chlamydospores formation was early and abundant. Chlamydospores of F.O.1 were ornamented, terminal or intercalary, single or in chains; whereas in F.O.3 and F.O.5 these were smooth and usually in chains. In isolate F.O.4 sporulation was poor. Size of the spores of the isolates have been presented in the table 1.

(b) *Effect of media on colony characters*

The colony characters showed difference in hyphal condition and pigment formation and no two isolates showed complete similarities. Isolates F.O.1 and F.O.5 could be distinguished from others by its long aerial hyphae. The mycelia of F.O.3 and F.O.4 were oppressed type. F.O.2 produced short aerial hyphae usually at the centre of the colony. The pigments produced by F.O.1 and F.O.5 were bright orange and that of F.O.3 and F.O.4 were either of dull or bluish to purple in colour. F O.2 produced dazzling white pigment in Martin's medium.

(c) *Effect of temperature on growth*

26°C was most suitable for growth of most of the isolates (F.O.1, F.O.2, F.O.4) while F.O.3 and F.O.5 attained its maximum growth at 32°C and 21°C respectively.

Table 1 *Measurement of the spores produced by the isolates of* F. oxysporum *f. sp.* carthami *on PDA plate at 21°C* (Mean of hundred spores)

F. O. 1 (IMI 186541)	F. O. 2 (IMI 204053)	F. O. 3 (IMI 204056)	F. O. 4 (IMI 186543)	F. O. 5 (IMI 186539)
Conidia				
1 Septate: 22.2 × 3.7μ	1 Septate: 20 × 3.7μ	1 Septate: 14 × 3.7μ	Aseptate: 11.1 × 4.5μ	1 Septate: 18 × 3.7μ
3 Septate: 32 × 5.5μ	2 Septate: 25 × 3.7μ	3 Septate: 28 × 3.7μ	1 Septate: 18.5 × 5.0μ	3 Septate: 22 × 3.7μ
5 Septate: 32 × 5.5μ	3 Septate: 32 × 3.7μ	5 Septate: 30 × 5.5μ		5 Septate: 30 × 5.5μ
				7 Septate: 44 × 5.5μ
Chlamydospore				
7.4 – 14.8μ	11.1 – 18.5μ	3.7 – 7.4μ	—	7.4– 18.5μ

Change of temperature affected the growth of F.O.1 maximum (14.7 and 24.2% reduction in growth rate at 32°C and 21°C respectively), but the growth of F.O.2 was least affected (5.4 and 13.7% reduction at 32°C and 21°C respectively).

F.O.1 and F.O.5 produced similar type of colony (sporadic, cottony, powdery at 32°C, 26°C, and 21°C respectively). In F.O.3 and F.O.4, the colonies were spreading and mat type at 32°C and 26°C respectively; whereas in F.O.2 they were mat and oppressed in appearance. At 26°C the growth rate of F.O.1 was the highest of all the isolates.

Discussion

Thus in the present study the five isolates of *F. oxysporum* f. sp. *carthami* were observed to vary among themselves in pathogenicity and growth rate, conidial and colony characters. In pathogenicity F.O.1 and F.O.2 represented the two extreme points; F.O.1 caused the maximum disease intensity while F.O.2 caused the minimum. The isolate F.O.5 showed much similarities with F.O.1, although some minor differences were there. F.O.3 and F.O.4 in pathogenicity and other morphological and cultural characters were intermediate of F.O.1 and F.O.2.

The aerial mycelia, rapid growth, large number of well septate macroconidia may help F.O.1 to dominate over the other isolates pathogenically. Armstrong et al. (1940), Wellman and Blaisdell (1941) reported that rapid radial growth and abundant aerial mycelia were known to be distinguishing features of virulent pathogens. Although absence of such correlation is quite common, yet it is apparently true for F.O.1 against F.O.2.

On the basis of the above mentioned characters, the isolates have been grouped into three physiologic variant groups: F.O.1 (IMI 186541) and F.O.5 (IMI 186539); F.O.2 (IMI 204053); F.O.3 (IMI 204056) and F.O. 4 (IMI 186539). Therefore, any effective control measures against the disease would require serious consideration of the physiologic races of *F. oxysporum* f. sp. *carthami*.

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