# Intra-population Variability in *Colletotrichum sublineoium* Infecting Sorghum

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## Abstract

Twelve monoconidial isolates were derived from a single-spore culture of *Colletotrichum sublineoium* isolate Cs 048 obtained from a local sorghum cultivar at Kovilpatti (India). The 12 isolates were evaluated for colony characteristics, spore morphology, and pathogenicity on six sorghum genotypes in the greenhouse. The isolates differed significantly in colony growth and morphology, and spore size within themselves and compared with the parental isolate. Variation in colony growth ranged from 0.1 % (Cs 048-7b-1) to 66.6% (Cs 048-1-2) relative to the parental isolate. Two of the 12 isolates showed distinct sectorial mycelial growth. Pathogenicity studies showed significant differences ($P < 0.001$) between isolates for latent period, virulence, aggressiveness, and virulence index. Isolate Cs 043-2 was most virulent infecting all six sorghum lines. Five other isolates were equally virulent infecting only two sorghum lines each. The parental isolate infected five sorghum lines. Cs 048-2 was the most aggressive isolate, while the parental isolate was moderately aggressive compared to the other isolates. The most virulent and aggressive isolates generally had the highest virulence index (virulence x aggressiveness x latent period$^{-1}$). The results indicated the inherent genetic variability between monoconidial isolates of C. *sublineoium,*

कोविलपट्टी (भारत) के स्थानीय ज्वार कृषिजोपजाति से पृथक्कृत कोलेटोट्राइकम सबलीनियोलमCS048 के एकल-बीजाणु से 12 एकल कोनिडी प्रथक्कृत प्राप्त किए गए। इन 12 पृथक्कृतों का पौध घर में कॉलोनी लक्षणता, बीजाणु आकारिकीय और ज्वार के छः जीन प्रारूपों पर रोग जनकता के लिए आंकलन किया गया। ये पृथक्कृत अपने जनक विभेद की तुलना में कालोनी वृद्धि और आकारिकिय तथा बीजाणु आकार में परस्पर भिन्न थे। जनक पृथक्कृत की तुलना में कॉलोनी वृद्धि की परिवर्तनता 0.1% (Cs048-7b-1) से लगाकर 66.6% (Cs048-1-2) तक थी। बारह में से दो पृथक्कृतों में सुस्पष्ट सैक्टरीय माइसिलियक वृद्धि परिलक्षित हुई। रोगजनकता अध्ययनों से इन पृथक्कृतों के आपस में अव्यक्त काल, उग्रता, आक्रमणशीलता और उग्रता अनुक्रमणिका में सार्थक भिन्नता (Pl 0.001) दृष्टि गत हुई। Cs 048-2 पृथक्कृत मुख्य रूप से उग्र होकर ज्वार की सभी छः जीन प्रारूपों को रोग ग्रसित किया। अन्य पाँच पृथक्कृतों में भी समान रूप से उग्रता देखी गई और इनमें से प्रत्येक ने दो-दो जीन प्रारूपों को रोग ग्रसित किया। जनक पृथक्कृत ने पाँच ज्वार के जीन प्रारूपों को रोग ग्रसित किया। Cs048-2 मुख्य रूप से अधिक आक्रमण कारी पृथक्कृत रहा, जबकि जनक पृथक्कृत अन्य पृथक्कृतों की तुलना में मध्यम रूप से आक्रमण कारी था। सामान्यतया, अधिकतम उग्रता और आक्रमण शीलता वाले पृथक्कृतों की उग्रता अनुक्रमणिका (उग्रता आक्रामणशीलता अव्यक्त काल) भी उच्चतम थी। इन परिणामों से सी. सबलीनियोलम बेके एकल कोनिडी पृथक्कृतों में वंशानुगत परिवर्तनशीलता का ज्ञान होता है।

Anthracnose of sorghum (*Sorghum bicolor* (L.) Moench), caused by *Colletotrichum sublineoium* Henn. Kabat *et Bub.* (Sutton, 1980) is widespread in warm, humid tropical and subtropical regions. The pathogen can infect the leaf, panicle and stalk leading to grain yield losses exceeding 50% (Harris *et al.,* 1964; Powel *et al,* 1977; Gorbet, 1977; Harris and Fisher, 1974; Mishra and Siradhana, 1979; Thomas *et al.,* 1996). Resistance to anthracnose in sorghum is governed by single dominant genes (Tenkouano and Miller, 1993) and therefore breeding for disease resistance may be an effective

means of controlling anthracnosc. Adequate knowledge of pathogen variability is required for analysis of virulence genes in the pathogen and identification of resistance genes in the host. Pathogenic variability in *C. graminicola* is known to be great and on the basis of differential reaction on 43 host genotypes, 44 races/pathotypes have so far been broadly identified (Nakamura, 1982; Ferreira and Casela, 1986; Ozolua *et al,* 1986; Ali and Warren, 1987; Cardwell *et at.,* 19S9; Pande *et ai,* 199I;Thakur, 1995; Thomas, 1995). Although sexual reproduction in the pathogen is not reported to occur in nature, the large genetic variation for pathogenicity is well known through asexual reproduction. In the present study, we have examined the extent of variability between monoconidial isolates of *C. graminicola* and discussed its possible implications in disease management through host plant resistance.

## Materials and Methods

The pathogen. The isolate of *C. sublineolum* (Cs 048) was obtained from local sorghum plant in Kovilpatti, India. Single-spore isolations were made by picking a single germinating conidium from water agar plates under a microscope with the help of a dummy- objective. These single-spore isolates were transferred to and grown on oatmeal agar plates at 25°C in continuous fluorescent light for 10 days. The cultures were observed for colony morphology, growth rate and spore morphology and classified in seven categories. Nine monoconidial isolates of Cs 048 selected from these seven different categories were used for further studies.

Growth characteristics and spore morphology. The nine monoconidial isolates (Table i) derived from Cs 048 were grown on oatmeal agar plates as previously described. Mycelial plugs (5mm dia), were removed from the advancing margins of 7-day-old cultures of each monoconidial isolate and transferred onto fresh oatmeal agar in petri dishes (90 mm dia). Three replicates of each isolate were maintained and incubated as previously described. The colony morphology (Huffy, smooth, sector) were assessed, and colony growth was assessed by measuring colony diameters using a ruler. Spore size (length and width) was also determined by mounting spores of each isolate in aniline blue-lactophenol and measuring them under a microscope (400x). A minimum of 100 spores were measured for each isolate. Growth sectors were observed in plates of isolates 048-1 and 048-7 (Fig. 1). These

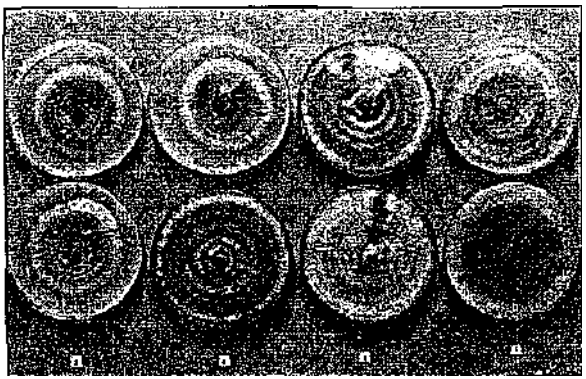

**Figure 1. Variation in colony characteristics of some monoconidial isolates of Colletotrichum graminicola from Kovilpatti: plates 1-3 of isolate Cs 048-1; plate 4 of isolate Cs 048-9; plates 5-6 of isolate Cs 048-7b, plate 7 of isolate Cs 048-4, and plate 8 of isolate Cs 048-8. Note the distinct sectorial growth in plates 2,3,5 and 6.**

were separated by picking hyphae from the sectors using a fine sterilized needle and placing them onto fresh oatmeal agar plates. The growth characteristics and pathogenicity of these sector-producing isolates were studied along with the nine isolates and the parental isolate.

Pathogenic variability. Six sorghum lines, selected from the International Sorghum Anthracnose Virulence Nursery (IS AVN), consisting of resistant (A 2267-2, IRAT 204), moderately resistant (IS 3094, IS 8354), and susceptible (IS 3089 and IS 18442) to C. sublineolum were used as potential differentials. Seed was surface sterilized with 0.1% HgCl2 for 4-5 min, washed thoroughly with distilled water and dried at room temperature (25° C) before sowing in an autoclaved black soil : sand : farmyard manure mix (3:2:2 by volume) in 18-cm square pots. Five plants were maintained in each pot. There were two replications (5 plants/replication) of each sorghum line for each isolate.

Inoculum and inoculation. The isolates were grown in 2% oatmeal broth for 5 days at 30°C and the conidia separated by filtering through muslin cloth. A concentration of $1x10^5$ conidia $ml^{-1}$ was prepared, using a hemocytometer, and two drops of Tween-20 were added to each suspension.

Plants of each line were spray inoculated at the 5-6 leaf stage (21-day old) with each of the nine isolates using a hand held sprayer. Potyethylene sheets were used to separate plants inoculated with different isolates. Following inoculations the plants were air-dried and then kept in a humidity chamber for 24 h. The plants were then transferred to greenhouse benches in a randomized block design, keeping isolates as blocks. Observations were taken for latent period (time in days from inoculation to appearance of first chlorotic/necrotic lesion) for each isolate-line combination.

Disease evaluation. Plants were scored for disease reaction 14 days after inoculations as R = resistant, no symptoms or chlorotic flecking; MR = moderately resistant, hypersensitive lesions, red spots or necrotic spots without acervuli; and S = susceptible, lesions with acervuli), and for disease severity on a 1-9 scale, where, 1 = no lesions; 2=1-5%; 3=6-10%; 4=11-20%; 5=21-30%; 6=31-40%; 7=41-50%; 8=51-75%; and 9= >75% leaf area covered with lesions.

Evaluation of pathogenicity and virulence. The nine monoconidial isolates tested earlier, along with the three sector-types and the parental culture (total 13 isolates) were inoculated on 21-day-old plants of the six sorghum lines following the method described above. One pot, consisting of five plants was used for each isolate-line combination. Each plant was taken as a replication. The experiment was repeated once.

Pathogenic variability was determined from the nature (compatible/incompatible) of host-pathogen interaction. Virulence of the isolates corresponded with disease reaction and was expressed as numerical values, where R = 1; MR = 2; and S= 3. Aggressiveness of the isolates corresponded with disease severity scores of 1-9. The virulence index of the isolates was determined as:

$$\text{Virulence index} = \text{Virulence} \times \text{aggressiveness} \times \text{latent period}^{-1}$$

Statistical analysis. Statistical analysis was done using GENSTAT (GENSTAT, 1986) and SAS (SAS, 1985). Analysis of variance was used to determine significant interactions of the isolate-line combinations. Groupings of the isolates and lines was done using general Linear Modeling with Duncan's multiple range test.

## Results

Morphological variation. Colony growth. The nine monoconidial isolates varied significantly from each other and from the parent isolate in their colony growth (Table 1). Three isolates (Cs 048-2, -3, and -4) had greater colony growth (102.4-103.2%) and five (Cs 048-1, -1-2, -1-2, -1-3, -5a, and -8) less colony growth (33.4- 97.9%) in relation to the parental isolate (100%). Three of the isolates (Cs 048-1-2, -1-3, and -7b-I) showed quite a distinct sectored mycelial growth patterns (Fig. 1).

Conidial size. Significant variations in conidial morphology (length and width) also occurred between the isolates (Table 2). Conidia of isolate Cs 048-3 were the shortest (22.6 x 3.8 µm) while of Cs 048-1 the largest (24.2 x 4,4 µm). Conidia of the remaining isolates were in the range of 22.7-23.8 x

**Table 1. Variation in colony growth of 12 monoeonidial isolates derived from a parental isolate, Cs 048 of Colletotrichum sublineolum from a local sorghum at Kovilpatti, India**

| Isolates designation | Colony diameter (mm) | Variation (%)[a] |
|---|---|---|
| Cs 048 (Parental) | 82.0 | 0.0 |
| Cs 048-1 | 50.1 | 33.3 |
| Cs 048-1-2[a] | 27.5 | 66.6 |
| Cs 048-1-3[a] | 51.5 | 37.4 |
| Cs 048-2 | 84.2 | 2.4 |
| Cs 048-3 | 84.7 | 3.0 |
| Cs 048-4 | 84.8 | 3.2 |
| Cs 048-5a | 80.3 | 2.3 |
| Cs 048-6 | 82.2 | 0.0 |
| Cs 04S-7b | 82.5 | 0.4 |
| Cs04S-7b-1[a] | 82.3 | 0.1 |
| Cs 048-8 | 80.5 | 2.! |
| Cs 048-9 | 82.8 | 07 |
| SE | +0.65 | |

[a]Growth sectors observed in the culture.
[b]Variation in relation to the parental isolate.

2.7-3.8 µm, and that of the parent isolate were 23.0 x 3.7 µm.

Pathogenic variation. Latent period. The mean latent period of the nine isolates on six sorghum lines varied from 2-7 days (Table 3). The isolate Cs 048-1-3 had the shortest mean latent period of 3 days across the lines, while Cs 048-6 had the longest, 5.4 days. On individual lines, the latent periods were shortest (3.1-4.3 days) on IS 3089 and longest (4.3-5.7 days) on A 2267-2. The differences were significant for isolates, host lines and their interactions (Table 4).

Virulence. The 13 isolates (12 monoeonidial, 1 parental) differed significantly (P<0.001) in virulence (Table 4). The isolate Cs 048-2 and the parental isolate Cs 048 induced a susceptible reaction on all six host lines, while the isolates Cs 048-9 and Cs 048-4 were pathogenic on four lines. The isolates Cs 048-6 and Cs 048-1-2 infected only three lines (Table 5).

Aggressiveness. Disease severity induced by isolates on host lines was quite variable, and was significantly different (P <0.001) between lines (Table 6). Based on the mean disease severity across lines, the 13 isolates were ciassified into six groups: (i) Cs 04S-2, Cs 048 (Parent) and Cs 4S-8; (ii) Cs 048-4; (iii) Cs 048-9 and Cs 04S-7b; (iv) Cs 04S-7b-1, Cs 048-1, Cs 048-1-3; (v) Cs 048-3, Cs 048-5a, and Cs 048-6; and (vi) Cs 048-1-2. Group (I) showed the highest mean disease severity across the lines while (iv) the lowest.

**Table 2. Variation in conidial size of nine monoconidial isolates derived from a single conidial culture of a Coltetotrichum sublineolum isolate ('Cs 04S)**

| Isolates designation | Length (µm) | | Width(µm) | |
|---|---|---|---|---|
| | Range | Mean | Range | Mean |
| Cs 048 (Parental) | 19.3-24.8' | 23.0±0.20 | 2.5-4.9 | 3.7±0.06 |
| Cs 04S-1 | 22.3-27.3 | 24.2+0.20 | 3.7-4.9 | 4.1±0.07 |
| Cs 048-2 | 19.8-27.3 | 23.6±0.22 | 2.5-3.7 | 3.7+0.03 |
| Cs 048-3 | 19.8-24.8 | 22.6±0.24 | 3.7-4.9 | 3.8±0.05 |
| Cs 048-4 | 19.8-26.0 | 23.2±0.19 | 3.7-4.9 | 3.8±0.04 |
| Cs 043-5a | 19.8-27.3 | 23.5±0.22 | 2.5-4.9 | 3.6±0.06 |
| Cs 048-6 | 22.3-26.0 | 23.8+0.16 | 2.5-4.9 | 3.3+0.09 |
| Cs 048-7b | 19.8-27.3 | 22.7±0.24 | 2.5-3.7 | 2.8+0.08 |
| Cs 048-8 | 21.1-27.3 | 23.7+0.20 | 2.5-4.9 | 3.6±0.03 |
| Cs 048-9 | 19.8-24.8 | 23.5+0.18 | 2.5-3.7 | 2.7±0.06 |
| SE | | +0.21 | | ±0.06 |

**Table 3. Latent period (days)[a] on six sorghum lines inoculated with monoconidial isolates derived from a *CoUetotrichum sublineoluin* isolate, Cs 048**

| | Sorghum lines | | | | | | |
|---|---|---|---|---|---|---|---|
| Isolate | A 2267-2 | IRAT 204 | IS 3758 | IS 8354 | IS 3089 | IS 18442 | Mean |
| Cs 048 (Parental) | 5.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.2 |
| Cs 048-1 | -[b] | 2.6 | 4.0 | 3.7 | 2.3 | 3.1 | 3.2 |
| Cs 043-2 | .1.9 | 17 | 4.0 | 4.0 | 4.0 | 4.0 | 3.9 |
| Cs 048-3 | - | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Cs 048-4 | - | 5.0 | 5.0 | 4.0 | 4.0 | 4.0 | 4.4 |
| Cs 048-5a | - | 7.0 | 4.0 | 4.0 | 4.0 | 4.3 | 4.7 |
| Cs 048-6 | - | 7.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.4 |
| Cs 048-7b | - | 5.0 | 4.0 | 4.0 | 5.0 | 5.0 | 4.6 |
| Cs 048-S | - | 5.0 | 5.0 | 5.0 | 5.0 | 4.7 | 4.9 |
| Cs 048-9 | - | 5.0 | 5.0 | 5.0 | *5.0* | 5.0 | 5.0 |
| Cs 048-1-2[c] | - | 3.3 | 4.0 | 3.0 | 3.0 | 3.0 | 3.3 |
| Cs 048-1-3[c] | - | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Cs048-7b-1[c] | - | 5.0 | 5.0 | 5.0 | 4.0 | 4.2 | 4.6 |
| Mean | 4.4 | 4.8 | 4.4 | 4.3 | 4.2 | 4.2 | |
| SD for isolate x host line interaction = 0.12 | | | | | | | |

[a]Mean of two experi menial runs
'No infection
[c]Isolates with growth sectors in culture

**Tnble 4. Analysis of variance for latent period, virulence, aggressiveness and virulence index of 13 *Colletotrichuin sublineoluin* isolates on six sorghum lines**

| Source of | Latent period | | Virulence | | Aggressiveness | | Virulence index | |
|---|---|---|---|---|---|---|---|---|
| . variation | df | MS | df | MS | df | MS | df | MS |
| IsolaCe | 12 | 17.94*** | 12 | 2.04*** | 12 | 8.00*** | (2 | 7.44"* |
| Host lines | 5 | 4.17**" | 5 | 29.63-** | 5 | 56.57*** | 5 | 31.17*** |
| Isolate x host line | 50 | 1.89*** | 60 | 0.76*** | 60 | 1.55"* | 50 | 1.31*** |
| Error | 356 | 0.14 | 413 | 0.10 | 419 | 0.38 | 353 | 0.31 |

**Table 5. Virulence (disease reaction) of 12 monoconidial isolates derived from a parental isolate of *Colletotrichum sublineolum* (Cs 048) on six sorghum lines**

| Isolate designation | Sorghum lines | | | | | |
|---|---|---|---|---|---|---|
| | A 2267-2 | IRAT204 | IS 3758 | IS 8354 | IS 3089 | IS 18442 |
| Cs 048 (Parental) | S | S | MR | S | S | S |
| Cs 048-2 | S | S | S | S | s | S |
| Cs 048-1-7b] Cs 043-1 -8] | R | S | s | MR | s | S |
| Cs 048-4 | R | MR | s | S | s | S |
| Cs 048-1-2[a]] Cs 048-6] | R | MR | MR | s | s | s |
| Cs 048-1] Cs048-1-3[a]] Cs 048-3] Cs 048-5] Cs 048-7b-t[a]] | R | MR | MR | MR | s | s |
| Cs 048-9 | R | R | S | S | s | S |

[a]Isolates with growth sectors in culture.

**Table 6. Aggressiveness (disease severity = 1-9 scale)[a] of 12 monoconidial isolates derived from a parental isolate ( Cs 048) of *Colletatrichum sublineolum* on six sorghum lines**

| Isolate designation | Sorghum lines | | | | | | |
|---|---|---|---|---|---|---|---|
| | A 2267-2 | IRAT204 | IS 3758 | IS 8354 | IS 3089 | IS 18442 | Mean |
| Cs CMS (Parental) | 2.0 | 2.0 | 2.0 | 3.4 | 3.4 | 5.2 | 3.0 |
| Cs 048-1 | 1.0 | 1.7 | 2.0 | 2.9 | 2.7 | 3.0 | 2.2 |
| Cs04S-2 | 2.1 | 2.3 | 2.6 | 2.7 | 3.6 | 4.1 | 2.9 |
| Cs 048-3 | 1.0 | 2.0 | 2.1 | 2.0 | 2.0 | 2.6 | 2.0 |
| Cs 048-4 | 1.0 | 3.0 | 3.1 | 3.0 | 4.6 | 5.0 | 3.3 |
| Cs 048-5a | 5.(1 | 2.0 | 2.3 | 2.1 | 1.9 | 2.6 | 2.0 |
| Cs 048-6 | 1.0 | 2.0 | 2.3 | 2.3 | 2.4 | 2.9 | 2.1 |
| Cs 04S-7b | 1.0 | 2.1 | 2.9 | 2.0 | 2.3 | 4.6 | 2.5 |
| Cs 048-8 | 1.0 | 2.4 | 3.0 | 2.7 | 3.3 | 5.0 | 2.9 |
| Cs 048-9 . | 1.0 | 2.0 | 2.0 | 2.1 | 2.4 | 4.6 | 2.4 |
| Cs 048-1-2[b] | 1.0 | 2.1 | 2.0 | 2.0 | 2.0 | 2.0 | 1.9 |
| Cs 048-1-3[b] | 1.0 | 2.0 | 2.0 | 2.0 | 2.2 | 3.6 | 2.1 |
| Cs 048-7b-1[b] | 1.0 | 2.0 | 2.0 | 2.0" | 3.4 | 3.4 | 2.3 |
| Mean | 1.2 | 2,1 | 2.4 | 2.4 | 2.E | 3.7 | |

SD for isolate x host line means = 0.47.

[a]Mean of two experimental runs, based on a 1.9 scale where 1 = no infection and 9 >75% leaf area covered with lesions.
Isolates with growth sectors in culture.

**Table 7- Virulence index[a] of 12 mono conidial isolates of *Colletotrichum sublineolum* on six host lines**

| Isolate designation | Sorghum lines | | | | | | |
|---|---|---|---|---|---|---|---|
| | A 2267-2 | IRAT204 | IS 3758 | IS 8354 | IS 3089 | IS 18442 | Mean |
| Cs 048 (Parental) | 1.2 | 1.1 | 1.0 | 2.6 | 2.6 | 3.9 | 2.1 |
| Cs 048-1 | b | 2.0 | 0.9 | 1.4 | 3.6 | 3.6 | 2.3 |
| Cs 048-2 | 1.6 | 1.7 | 1.9 | 2.0 | 2.7 | 3.1 | 2.2 |
| Cs 048-3 | - | 0.9 | 0.9 | 0.8 | 1.0 | 1.5 | 1.0 |
| Cs 048-4 | - | 1.3 | 1.9 | 2.2 | 3.3 | 3.8 | 2.5 |
| Cs 048-5a | - | 0.6 | 1.4 | 1.2 | 1.5 | 1.8 | f.3 |
| Cs 04S-6 | - | 0.2 | 1.1 | 1.2 | 1.5 | 1.7 | 1.2 |
| Cs048-7b | - | 1.1 | 1.8 | 1.0 | 1.3 | 2.7 | 1.6 |
| Cs 048-8 | - | 1.3 | 1.6 | 1.0 | 1.9 | 3.1 | 1.8 |
| Cs 048-9 | - | 1.0 | 1.2 | 1.0 | 1.3 | 2.7 | 1.5 |
| Cs 048-1-2[c] | - | 1.4 | 1.0 | 1.7 | 2.0 | 2.0 | 1.6 |
| Cs 048-1-3[c] | - | 1.3 | 1.3 | 1.3 | 2.2 | 3.6 | 2.0 |
| Cs 048-7b-1[c] | - | 0.8 | 0.8 | 0.8 | 2.6 | 2.5 | 1.5 |
| Mean | 1.4 | J.J | 1.3 | J.4 | 2.1 | 2.8 | |

SD for isolate *x* host line means = 0.37

[a] [(disease reaction *x* disease severity) *x* latent period$^{-1}$)
[b]Considered as avirutenl since no infection developed
[c]solates with growth sectors in culture

Similarly, the six host lines were classified into four groups on the basis of disease severity, ranging from highly susceptible to resistant: (1) IS 18442, (ii)IS 3089,IS 3758,(iii)IS 8354,IRAT204, (iv) A 2267-2. The isolate x line interactions were also highly significant ($P < 0.001$) (Table 4).

Virulence index. Virulence index was significantly different between isolates (Table 4). The parental isolate Cs 048 had the highest virulence index (3.9) on line IS 18442, followed closely by Cs 048-4 (3.8) (Table 7). However, the isolate Cs 048-4 had the maximum mean virulence index (2.5) across host lines whereas the isolate Cs 048-3 had the minimum (1.0). Among the host lines, the most susceptible was IS 18442 (virulence index 2.8). The most resistant line was IRAT 204 (virulence index 1.1).

Correlation matrix. Virulence index was negatively correlated with latent period and positively correlated with virulence and aggressiveness. There was no relationship between aggressiveness and latent period, although aggressiveness was positively correlated with virulence (Table 8).

**Table 8. Correlation coefficient between different variables (DF : 423/424)**

| | Latent period | Virulence | Aggressiveness |
|---|---|---|---|
| Virulence | -0.15*** | | |
| Aggressiveness | -0.02 | 0.66*** | |
| Virulence index | -0.45*** | 0.65*** | 0.81*" |

** and *** significant at 1 and 0.1%, respectively.

## Discussion

The monoconidtal isolates of *C. sublineolum* derived from a single-conidium parental stock culture differed significantly for cultural characteristics, conidial morphology and pathogenicity. These results indicate great potential for genetic variability that exists within *C. sublineolum* popula-

tions. Intra-population variation in pathogenicity between sister conidial lines from single lesion and monoconidial cultures have been reported in other pathogens, including *Pyricularia oryzae* (Ou and Ayad, 1968) and *Fusarium spp.* (Sutton, 1980). The possible mechanism of genetic changes within population of *P. oryzae* have been demonstrated due to parasexuaiity and heterocytosome (Yamasaki and Niizeki, 1963; Fatemi and Nelson, 1977). The situation in C. *subtineolum* may be different from these, as the conidia of C. *sublineolum* are single-celled and the information on the nature and the number of nuclei per conidium is not known.

Formation of clear growth sectors in colonies of C. sublineolum grown on oatmeal agar is thought to be the first report in this fungus. Two of the three isolates (Cs 048-1 -2 and -1 -3) with sectored growth also had slower colony growth (Table 1) indicating some growth suppression. These two isolates had relatively short latent periods (Table 3), but lower virulence indices (Table 7). Two of these growth sector isolates Cs 048-1-3 and Cs 048-7b-l were different from the original isolates Cs 048-1 and Cs 048-7b, respectively. However, the mechanism of this variability is not clear. The parental isolate Cs 048 was the most pathogenic infecting more lines than the daughter monoconidial isolates.

We have used the virulence index to measure the quantitative pathogenicity of the isolates. Although the virulence index had a strong negative correlation with the latent period and a positive correlation with virulence and aggressiveness, there were some exceptions. For example, the isolate Cs 048-8 had a moderate latent period and aggressiveness, but a virulence index as high as the most aggressive and virulent isolate Cs 048-2 on host line IS 18442.

Further studies on intra-population variability in *C. sublineolum,* including nuclear staining and an investigation of nuclear behavior during spore-germination, and rDNA sequence analysis (Sherriff *et al.,* 1995) of the monoconidial isolates may help elucidate the origin and extent of pathogenic variability within and between populations of *C. sublineolum.* This would be useful in understanding the genetics of host-pathogen interaction, which is important to the successful incorporation and deployment of anthracnose resistance.

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# Spines, a New Avenue for infection by *Alternaria carthami* on Safflower

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**Abstract**

Spines present on the leaf margin in safflower (*Carthamus tinctorius*) cultivars were observed to be a new site of infection by the fungal pathogen *Alternaria carthami.* The infection through spines took place whenever the pathogen was seed borne and serve as a primary source of inoculum. An opening dia of 120 µ at individual spines apex was a pre-requisite to cause infection through spines. The opening at spines apex varied as per the location of spine on the leaf margin and in general, increase at the rate of 7 µ/day, till it reaches 120 p.. Therefore, spines at different locations on leaf margin varied in their infection time.

कुसुंभ (*कार्थेमस टींक्टोरियस*) की कृषिजोपजातियों में पत्तियों की कोर पर अवस्थित शूल कवक रोगजनक *एल्टरनेरिया कार्थेमी* के संक्रमण के लिए एक नवीन साधन प्रेक्षित हुआ। जब जब भी रोग जनक बीजोढ़ रहा और निवेश द्रव्य का मुख्य स्रोत रहा तब शूल द्वारा संक्रमण हुआ। शूलों द्वारा संक्रमण होने के लिए कम से कम 120u व्यास का प्रत्येक शूल शीर्ष पर मुँह होना प्राथमिक आवश्यकता थी। शूलों के शीर्ष का मुँह के आकार में विभिन्नता पत्ती पर शूलों के स्थान निर्धारण पर आधारित थी, और सामान्यतया यह 7u प्रतिदिन के रूप में बढ़ती रहती है और अन्ततः 120u आकार में आ जाती है। इसीलिए भिन्न भिन्न स्थानों पर निर्धारित शूलों की संक्रमणता काल में भिन्नता देखी गई।

Natural openings, such as stomata (Berry, 1959), lenticels (Allen, 1957), hydathodes (Baker *et al.,* 1954) and wounds (Otieno, 1962) have been reported to serve as avenues of penetration for pathogens. *Alternaria carthami* causing leaf spot disease in safflower was noticed to cause infection of leaf margin at the site of spines located on the leaf margin whenever the pathogen was externally seed borne. As there is no report of spine to serve as natural avenue of penetration and infection, various aspects relating to infection through spines in safflower were investigated.

## Materials and Methods

Seed samples of 32 safflower cultivars of spiny genotypes, carrying 86% externally seed borne infection of *A. carthami* (Borkar and Shinde, 1989) were used for pot experiments. The plants were raised under glasshouse conditions to avoid the airborne secondary inoculum of *A. carthami.*

To study the cellular structure of the spines, the healthy spiny leaves of safflower were treated with 10% NaOH solution (Foster, 1965) for 48 h to remove chlorophyll content of the spines and were observed under microscope. The leaves of spiny cultivars of safflower carrying infection or appeared to carry infection of *A. carthami* on spines, were also treated with 10% NaOH, as above, to facilitate the histopathological and micro-metrical studies of spines during infection.

To confirm the infection through spines, the tip of the spines of fully grown safflower leaves were