



## Cultural variability in *Colletotrichum gloeosporioides* Penz infecting major fruit crops in Khandesh region of Maharashtra

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### Abstract

*Colletotrichum gloeosporioides* (Penz.) Penz and Sacc, causal organism of anthracnose disease in pomegranate, sweet orange, banana and guava. The isolates of *C. gloeosporioides* designated as CgPD 1, CgPJ 2, CgPN 3, CgSD 4, CgSJ 5, CgSN 6, CgBD 7, CgBJ 8, CgBN 9, CgGD 10, CgGJ 11 and CgGN 12. The diseased sample collected from four different fruit crops and three different district in Khandesh region of Maharashtra. These isolates were characterized for cultural variation. The culture of isolate CgPD 1 recorded maximum average growth rate of 12.85 mm/day while, isolate CgBN 9 recorded lowest growth with 9.15 mm/day. Most of the isolates shows excellent sporulation. The colony colour of isolate shows white, pinkish, creamy white and white to dull white. Most of the isolates showed raised fluffy, regular smooth growth. The pigmentation varied from white, pinkish and white to orange. Among the five culture media (one synthetic and four non synthetic) media tried, the maximum growth of *C. gloeosporioides* irrespective of isolates was obtained on Potato Dextrose Agar (7.48 mm day<sup>-1</sup>). Host Leaf Extract Agar and Oat Meal Agar medium were also statistically significant followed by with Potato Dextrose Agar and recorded average growth rate of 5.98 mm/day and 5.19 mm/day, respectively. The Peptone Rose Bengal Agar medium was found in poor growth of isolates (3.77 mm/day).

**Keywords:** *Colletotrichum gloeosporioides*, Khandesh, major

### Introduction

*Colletotrichum gloeosporioides* (Penz.) Penz and Sacc, is an important pathogen causing anthracnose disease in pomegranate, sweet orange, banana and guava fruit crops. It is very difficult to manage it due to its broad host range, extreme variability in pathogenic isolates and prolonged active phase of the disease cycle. Eight isolates of *Colletotrichum gloeosporioides* collected from northern Karnataka (India) Showed significant difference in relation to radial growth, colony character, pigmentation and sporulation on potato dextrose agar. Maximum radial growth of 90.0 and 88.5 mm was recorded in Cgr-2 and Cgr-1 isolates, respectively (Prashanth and Sataraddi, 2011) [6]. Studies on cultural variability of the pathogen are of immense use in understanding the nature of pathogen. *Colletotrichum* species are cosmopolitan and it has been shown that multiple species can infect a single host while single species can infect multiple host (Cai *et al.*, 2009) [3]. *Colletotrichum* species may adapt to new environments (Sanders and Korsten, 2003) [8] leading to serious cross infection problems in plant production. The study of pathogenic variability of *Colletotrichum* species is therefore important. Hence, present investigation was undertaken with the objective to study cultural variability of *C. gloeosporioides* (Penz) collected four different fruit from three different districts in Khandesh region of Maharashtra state.

### Material and methods

#### Collection of diseased samples

Diseased specimens from different fruit crops in the form of infected fruits, were collected on the basis of symptoms from different districts in Khandesh regions in the Maharashtra state by personal visit.

#### Isolation of the pathogen

Pomegranate, sweet orange, banana and guava fruits showing typical symptoms of anthracnose collected from different districts of Khandesh region in Maharashtra state during June - August 2018. Standard tissue isolation procedure was followed to isolate the pathogen. The infected tissues along with healthy portions were surface sterilized with 0.1 per cent mercuric chloride solution for 30 seconds and such bits were transferred to petridishes containing sterile water successively for three times and then bits were transferred into petridishes containing 15 ml of Potato Dextrose Agar medium and incubated at 28°C for 7 days. Pure culture of the fungus was obtained by single spore isolation.

#### Pathogenicity

To prove the pathogenicity of isolated pathogen's detached fruit inoculation technique was adopted for pomegranate, sweet orange, banana and guava fruits. The inoculation on

detached fruit could infect the pomegranate, sweet orange, banana and guava fruits and cause disease symptoms. The symptoms on fruit appeared within 3-6 days, whereas no symptoms appeared on the uninoculated fruits. Symptom development was rapid in banana fruits (72-90 hrs) followed by sweet orange (105 hrs). However, symptom initiation was comparatively late in pomegranate and guava (115 hrs).

Reisolations were made from every inoculated fruit to compare the test fungus. All isolates fulfilled the requirements of Koch's postulates. Therefore, all isolates fulfilled the pathogenicity test were carried and used for the further study.

### Identification of pathogen

The pathogen was identified as *Colletotrichum gloeosporioides* Penz. Based on its mycelial, conidial characteristics through standard mycological keys (Barnett *et al.*, 1972)<sup>[2]</sup> and coded as follows

### Cultures of *C. gloeosporioides*

| Sr. No | Isolate No. | District  | Host         |
|--------|-------------|-----------|--------------|
| 1      | CgPD 1      | Dhule     | Pomegranate  |
| 2      | CgPJ 2      | Jalgaon   |              |
| 3      | CgPN 3      | Nandurbar |              |
| 4      | CgSD 4      | Dhule     | Sweet orange |
| 5      | CgSJ 5      | Jalgaon   |              |
| 6      | CgSN 6      | Nandurbar |              |
| 7      | CgBD 7      | Dhule     | Banana       |
| 8      | CgBJ 8      | Jalgaon   |              |
| 9      | CgBN 9      | Nandurbar |              |
| 10     | CgGD 10     | Dhule     | Guava        |
| 11     | CgGJ 11     | Jalgaon   |              |
| 12     | CgGN 12     | Nandurbar |              |

### Maintenance of the isolates

Different isolates of *C. gloeosporioides* Penz. Were maintained separately on PDA slants and later sub cultured on sterilized petriplates containing Potato Dextrose Agar medium for further study.

### Variation in cultural characters

#### Growth on solid media

The growth characters of *C. gloeosporioides* were studied on five solid media *viz.*, Potato Dextrose Agar, Oat Meal Agar, Peptone Rose Bengal Agar, Host Leaf Extract Agar, Richards's Agar. All the media were sterilized at 1.1 kg/cm<sup>2</sup> pressure for 15 minutes. Twenty ml of each of the medium was poured in each petriplate separately. Such petriplates were aseptically inoculated with 5 mm disc cut-outs from periphery of actively growing culture and incubated at 28°C for the period of 7 days. Each treatment was replicated three times. Observations were taken 7 days after inoculation. The mycelial colour, substrate colour, margin of the colony, topography, centre of the colony, sporulation and spore size were recorded at 28°C for 7 days after inoculation. The data on radial growth was analyzed statistically.

The sporulation was graded relatively as Excellent, Good, Moderate and Poor sporulation based on conidia / microscopic

field as detailed below table.

| Sr. No | Score | Grade     | Conidia/ microscopic field (400x) |
|--------|-------|-----------|-----------------------------------|
| 1      | ++++  | Excellent | >75                               |
| 2      | +++   | Good      | 50-75                             |
| 3      | ++    | Moderate  | 25-50                             |
| 4      | +     | Poor      | 1-25                              |

### Result and Discussion

#### Variation in cultural characters

##### Growth rate on PDA

The data presented in the table 1 revealed that, *C. gloeosporioides* isolates exhibited high degree of variation in growth on PDA. Maximum average growth rate of 12.85 mm/day was observed in isolates *Colletotrichum gloeosporioides* Pomegranate Dhule (CgPD 1) and it was statistically at par with isolates CgPJ 2, CgPN 3, CgSD 4, CgSN 6 and CgSJ 5. These isolates were referred as fast growing isolates.

Isolates CgBD 7, CgGD 10, CgGJ 11 and CgGN 12 ranked second in the growth rate and were on par with each other. These isolates were referred moderately fast growing, as compared to slow growing isolates CgBJ 8 and CgBN 9.

The isolate *Colletotrichum gloeosporioides* Banana Nandurbar (CgBN 9) was slow growing with average growth rate of 9.15 mm/day. Similar trend in colony morphology was reported by Abhang *et al.* (2002). (Table 1).

**Table 1:** Variation in average growth rate and sporulation of *C. gloeosporioides* isolates on PDA.

| Isolates | Growth rate (mm /day) |       |       | Growth      | Sporulation |
|----------|-----------------------|-------|-------|-------------|-------------|
|          | R I                   | R II  | Mean  |             |             |
| CgPD 1   | 12.88                 | 12.82 | 12.85 | Fast growth | ++++        |
| CgPJ 2   | 12.81                 | 12.60 | 12.71 | Fast growth | ++++        |
| CgPN 3   | 12.72                 | 12.65 | 12.68 | Fast growth | ++++        |
| CgSD 4   | 12.75                 | 12.67 | 12.71 | Fast growth | ++++        |
| CgSJ 5   | 12.69                 | 12.45 | 12.57 | Fast growth | +++         |
| CgSN 6   | 12.62                 | 12.78 | 12.70 | Fast growth | ++          |
| CgBD 7   | 9.89                  | 9.60  | 9.74  | Moderate    | ++++        |
| CgBJ 8   | 9.28                  | 9.28  | 9.28  | Slow        | +++         |
| CgBN 9   | 9.14                  | 9.16  | 9.15  | Slow        | +++         |
| CgGD 10  | 11.67                 | 11.48 | 11.57 | Moderate    | ++++        |
| CgGJ 11  | 11.62                 | 11.84 | 11.73 | Moderate    | +++         |
| CgGN 12  | 11.30                 | 11.51 | 11.40 | Moderate    | ++++        |
| SE ±     |                       |       | 0.09  |             |             |
| CD @ 5%  |                       |       | 0.27  |             |             |

Whereas, Excellent (++++), Good (+++), Medium (++) , Poor (+)

##### Sporulation on PDA

Maximum sporulation was observed in *Colletotrichum gloeosporioides* Pomegranate Dhule (CgPD 1) and it was statistically superior over rest of the isolates. This was followed by CgPJ 2, CgPN 3, CgSD 4, CgBD 7, CgGD 10 and CgGN 12. Sporulation ability was found to be low and there was not significant difference in isolates CgSJ 5, CgBJ 8, CgBN 9 and CgGJ 11. Extremely low sporulation was observed in isolates *Colletotrichum gloeosporioides* Sweet orange Nandurbar (CgSN 6). (Table 1).

**Table 2:** Variation in the colony morphology of *C. gloeosporioides* isolates

| Sr. No | Isolates | Colony colour       | Substrate colour | Topography     | Pigmentation    | Margin    | Zona- tion | Sporu -lation |
|--------|----------|---------------------|------------------|----------------|-----------------|-----------|------------|---------------|
| 1      | CgPD 1   | White               | Orange to black  | Raised fluppy  | White           | Regular   | Yes        | ++++          |
| 2      | CgPJ 2   | Creamy white        | Orange to black  | Flat           | White           | Irregular | No         | ++++          |
| 3      | CgPN 3   | White               | Whitish to black | Fluppy flat    | White           | Regular   | No         | ++++          |
| 4      | CgSD 4   | White to light gray | Orange           | Raised fluppy  | White           | Regular   | No         | ++++          |
| 5      | CgSJ 5   | White to dull white | Orange           | Flat           | White to orange | Irregular | No         | +++           |
| 6      | CgSN 6   | White               | Orange           | Raised fluppy  | White to orange | Irregular | Yes        | ++            |
| 7      | CgBD 7   | Pinkish             | Pinkish red      | Flat           | Pinkish         | Regular   | No         | ++++          |
| 8      | CgBJ 8   | Pinkish             | Pinkish red      | Flat           | Pinkish         | Regular   | No         | ++++          |
| 9      | CgBN 9   | Pinkish             | Pinkish red      | Raised fluppy  | Pinkish         | Irregular | No         | ++++          |
| 10     | CgGD 10  | White               | Orange           | Flat to raised | White           | Regular   | No         | ++++          |
| 11     | CgGJ 11  | White to orange     | Orange           | Raised fluppy  | Orange          | Irregular | Yes        | +++           |
| 12     | CgGN 12  | White               | White            | Raised Fluppy  | White           | Regular   | No         | ++++          |

Whereas, Excellent (++++), Good (+++), Medium (++) , Poor (+)

### Growth characters on PDA

There was greater variation in the colony morphology within *C. gloeosporioides* isolates. The colony colour was the most variable factor and none of the isolate was found to be identical with each other. The colonies of all isolates were initially white, gaining differential colour shades upon aging. The most predominant colour was white with various intermediate shades. The colony colour of CgPD 1, CgPN 3, CgSJ 5, CgSN 6, CgGD 10 and CgGN 12 were white, colony of CgPJ 2 were creamy white, colony of CgGD 11 were white to orange, colonies of CgSD 4 was white to light gray, with advancing radial growth of white colour. In all other isolates CgBD 7, CgBJ 8 and CgBN 9, the colony colour was pinkish. After seven days of incubation there was pigmentation of acervuli in the form mass of white encrustations regularly observed in CgPD 1, CgPJ 2, CgPN 3, CgSD 4 and CgGN 12. Similarly, white to orange coloured mass of conidia was found to be scattered in the cultures of CgSJ 5 and CgSN 6. While pinkish colour mass of conidia were observed in CgBD 7, CgBJ 8 and CgBN 9 isolates. The present findings are in collaboration with Prashanth (2007) [5] according to them variation in colour and pigmentation might be due to different biotic and abiotic stresses.

Isolate wise colony texture in details found to be. Entirely uniform and regular colony growth was observed in isolates CgPD 1, CgPN 3, CgSD 4, CgBD 7, CgBJ 8, CgGD 10 and CgGN 12, whereas, irregular and uneven colony growth was found in isolate of CgPJ 2, CgSJ 5, CgSN 6, CgBN 9 and CgGJ 11.

On the basis of colony texture, *C. gloeosporioides* grouped as flat, raised fluppy and flat to fluppy. Isolates CgPJ 2, CgSJ 5, CgBJ 7 and CgBJ 8 represent the flat colony texture. Isolates CgPD 1, CgSD 4, CgSN 6, CgBN 9, CgGJ 11 and CgGN 12 fall in raised fluppy, while isolates CgPN 3 and CgGD 10 were categorized as flat to fluppy colony texture. The researcher such as Sangdee *et al.* (2011) on *C. capsici* and Hartman and Wang (1990) on *C. capsici* and *C. gloeosporioides* have done work on the cultural variability of *Colletotrichum* spp.

### Variation in growth of *C. gloeosporioides* isolates as influenced by culture media

Data presented in Table 3 revealed that the treatment differences due to interaction between *C. gloeosporioides*

isolates and different media tried were statistically significant. The average growth rate of *C. gloeosporioides* irrespective of isolates and media was 5.48 mm per day with the range of 8.83 mm /day *Colletotrichum gloeosporioides* Pomegranate Nandurbar (CgPN 3) to 2.57 mm /day *Colletotrichum gloeosporioides* Pomegranate Jalgaon (CgPJ 2). The maximum growth rate of 8.83 mm /day (CgPN 3) and it was statistically at par with the growth rate of CgPD 1. These isolates were grouped as fast growing isolates. The second group of isolates CgPJ 2 and CgSD 4 with average growth rate ranging between 7.05 – 5.95 mm day<sup>-1</sup>. The third group having growth rate < 5.95 but > 5.48 (average) mm day<sup>-1</sup> include isolates CgSJ 5. All other remaining isolates showed lesser growth rate than average (5.48 mm day<sup>-1</sup>). All the above isolates were from different host sources. Isolates from banana were found in poor growth rate than isolated from other fruit crops. The lowest average growth rate was attended in CgBN 9 (4.39 mm day<sup>-1</sup>) from banana.

Among the five culture media (one synthetic and four non synthetic) media tried, the maximum growth of *C. gloeosporioides* irrespective of isolates was obtained on Potato Dextrose Agar (7.48 mm day<sup>-1</sup>). Host Leaf Extract Agar and Oat Meal Agar medium were also statistically significant followed by with Potato Dextrose Agar and recorded average growth rate of 5.98 mm /day and 5.19 mm /day, respectively. This was followed by growth rate on Richard's Agar and Peptone Rose Bengal Agar medium. In all these isolates, Potato Dextrose Agar and Host Leaf Extract Agar the growth rate was more than the average growth rate. The lowest average growth rate was recorded on Peptone Rose Bengal Agar 3.77 mm /day.

The growth of most of the isolates on Potato Dextrose Agar and Richard's medium was profuse, filamentous and typical as per the description of the growth of the test organism under study and as per the cultural characters described earlier. The growth of isolates on Oat Meal Agar showed excessive development of orange coloured spore masses in all isolates of *C. gloeosporioides*.

The interaction between media and isolates was statistically highly significant. Isolates CgPD 1, CgPJ 2, CgPN 3, CgSD 4, CgSJ 5, CgSN 6, CgGD 10, CgGJ 11 and CgGN 12 attended maximum growth on Potato Dextrose Agar, isolate CgPD 1, CgPJ 2 and CgPN 3 on Richard's Agar, isolate CgPD 1 and CgSJ 5 on Peptone Rose Bengal Agar, isolate CgPD 1, CgPJ

2, CgPN 3, CgSD 4, CgSJ 5, CgSN 6, CgBD 7, CgBJ 8 and CgGN 12 on Host Leaf Extract Agar, isolate CgPD 1, CgPJ 2, CgPN 3 and CgSD 4, on Oat Meal Agar. All these interactions were superior over rest of the interactions recorded of average growth rate of 5.48 mm /day. The lowest growth interaction was found in the interaction between CgPJ 2 and Peptone

Rose Bengal Agar 2.57 mm /day. This indicated that isolates differ in their ability to grow on nutritionally different substrates.

These result were similar with Reddy (2000)<sup>[7]</sup>, Saxena (2002)<sup>[10]</sup> and Thangmani *et al.* (2011).

**Table 3:** Variation in growth of *C. gloeosporioides* isolates as influenced by different culture media (in mm / day)

| Isolates         | PDA  | Richard's Agar | Peptone | Rose Bengal Agar | Host Leaf Extract Agar | Oat Meal Agar | Mean |         |
|------------------|------|----------------|---------|------------------|------------------------|---------------|------|---------|
| CgPD 1           | 8.80 | 8.03           | 7.13    | 6.70             | 5.97                   | 7.33          |      |         |
| CgPJ 2           | 8.63 | 7.93           | 2.57    | 6.13             | 6.03                   | 6.26          |      |         |
| CgPN 3           | 8.83 | 8.17           | 2.90    | 6.63             | 5.60                   | 6.43          |      |         |
| CgSD 4           | 8.63 | 4.23           | 3.10    | 6.57             | 7.83                   | 6.07          |      |         |
| CgSJ 5           | 8.33 | 3.77           | 5.87    | 6.00             | 4.80                   | 5.75          |      |         |
| CgSN 6           | 8.37 | 3.70           | 3.27    | 6.70             | 5.07                   | 5.42          |      |         |
| CgBD 7           | 5.23 | 4.47           | 3.37    | 5.93             | 5.10                   | 4.82          |      |         |
| CgBJ 8           | 5.13 | 4.17           | 3.70    | 5.63             | 4.47                   | 4.62          |      |         |
| CgBN 9           | 4.83 | 4.13           | 3.67    | 5.23             | 4.10                   | 4.39          |      |         |
| CgGD 10          | 7.80 | 3.80           | 3.10    | 5.13             | 4.07                   | 4.78          |      |         |
| CgGJ 11          | 7.93 | 3.63           | 3.47    | 5.33             | 4.10                   | 4.89          |      |         |
| CgGN 12          | 7.23 | 3.70           | 3.10    | 5.70             | 5.10                   | 4.97          |      |         |
| Mean             | 7.48 | 5.19           | 3.77    | 5.98             | 4.98                   | 5.48          |      |         |
|                  |      |                |         |                  |                        |               | SE ± | CD @ 5% |
| Isolates         |      |                |         |                  |                        |               | 0.03 | 0.08    |
| Media            |      |                |         |                  |                        |               | 0.01 | 0.03    |
| Isolates X Media |      |                |         |                  |                        |               | 0.10 | 0.29    |

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