Isolation and disease scoring of *Colletotrichum lindemuthianum* causing anthracnose disease in French bean

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**Abstract:** The experiment on “Isolation and disease scoring of *Colletotrichum lindemuthianum* causing anthracnose disease in French bean (*Phaseolus vulgaris* L.)” was carried out by planting Forty frenchbean accessions. Two resistance genotypes viz., D-line, L-line and two susceptible varieties viz., Kanchana and Jwala were used for evaluation. Thirty days plants were inoculated with *Colletotrichum lindemuthianum* spore culture and disease scoring was done using 0-9 scale, 15 days after inoculation, genotypes were rated as highly resistant, resistant, moderately resistant, susceptible and highly susceptible based on disease severity. The genotypes EC500474, IC328848, EC530819, EC500641, IC319370 and EC398483 showed highly resistance for the *C. lindemuthianum* pathogen under field conditions, along with the confirmed resistant lines D-line and L-line. The genotypes EC115962, EC540876, EC531078 and IC318852 showed high susceptibility for the pathogen along with the susceptible checks Kanchana and Jwala.

**Key words:** Anthracnose (*Colletotrichum lindemuthianum*), French bean (*Phaseolus vulgaris*), Disease scoring

**Introduction**

French bean is a versatile crop, can be grown in all types of soils ranging from light sandy loam to clay soils but it cannot withstand water logging. French bean belongs to family Fabaceae. It is an annual herb with an erect and bushy growth (20-60 cm tall) or twining with stems of 2-3 m long with a tap root and nitrogenous nodules. The highest yields are obtained in soils with a pH of 5.3 and 6.0 coupled with cool climate (Singh and Chauhan, 2009). It is the third most important legume crop grown worldwide, superseded by *Glycine max* (soybean) and *Arachis hypogaea* (peanut), but first in direct human consumption (Broughton et al., 2003). Common beans provide an inexpensive food stuff rich in macronutrients viz., protein, starch and micronutrients such as Iron, Calcium, Magnesium, Phosphorus and Zinc. It is also rich in number of other stored bioactive compounds such as phytates, polyphenols, tannins, raffinose saccharides, lectins, protease, α-amylase inhibitors and saponins with positive health implications through their antioxidant, anti-tumour or phyto-oestrogenic activity (Doria et al., 2012). Bean anthracnose caused by *C. lindemuthianum* (Sacc. & Magn.) is the most widespread and destructive disease of common bean in tropical and subtropical areas of the world, mainly under cool and humid conditions. Yield loss due to bean anthracnose can reach up to 100% when contaminated seeds are planted, and prolonged conditions favorable to disease development occur during the crop season (Pastor-Corrales and Tu, 1989; Kelly et al., 1994 and Sharma et al., 1994). Anthracnose caused by the fungus *Colletotrichum lindemuthianum* (Sacc. and Magn.) is known to be the most predominant disease and it is a seed borne and highly destructive in all bean growing countries of the world. The pathogen can attack leaves, stems, pods, seeds, and causes a yield loss of 90-100% in many bean growing regions throughout the world (Sutton, 1992; Pastor-Corrales and Tu, 1989; Tu, 1992a; Kelly et al., 1994). The disease can affect all aerial parts of the bean plant and the infected cotyledons exhibit circular dark brown to black lesions. The bean leaves show dark brown necrotic lesions and there will be decrease in leaf photosynthetic activity (Bassanezi et al., 2001).

Pod infection appears as necrotic sunken lesions that develop into sunken cankers. Young pods may shrivel or dry when severely attacked by the fungus. The fungus can invade the pod surface and infect the seed coat and cotyledon of the developing seeds. Infected seeds are often discolored, shriveled and contain dark brown to black cankers. Complete yield loss can occur with susceptible genotypes when conditions are favorable for the pathogen during growing season (Gonzalez et al., 1998). Seed infection results in the introduction of the disease into new areas or new races into new geographic regions (Chen et al., 2007). The main objective of present study is to isolate the genotypes showing resistance to anthracnose disease at field level with high yielding capability under good agronomic practices.

**Materials and Methods**

Molecular work was conducted in the Molecular Biology laboratory, Department of Plant Biotechnology and *Colletotrichum* pathogen isolation was carried out in the Plant Pathology Laboratory, University of Agricultural Sciences, GKVK, Bengaluru.

**Isolation of the pathogen -**

**Collection of diseased specimen:** The leaves and pods of French bean showing typical symptoms of anthracnose with numerous pin point sunken black dots infected by *C. lindemuthianum* were collected from the field.

**Microscopic observation:** The infected specimen was microscopically examined for confirmation of the fungus. The diseased tissue was teased with a sharp blade on a glass slide with
Isolation and disease scoring of anthracnose disease

Table 1: Scoring for the disease reaction

<table>
<thead>
<tr>
<th>Scale</th>
<th>Disease reaction</th>
<th>Disease severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Immune</td>
<td>No infection</td>
</tr>
<tr>
<td>1</td>
<td>Highly resistant</td>
<td>Less than 1% leaf area damaged</td>
</tr>
<tr>
<td>2</td>
<td>Resistant</td>
<td>1 – 5% leaf area damaged</td>
</tr>
<tr>
<td>5</td>
<td>Moderately resistant</td>
<td>6 – 25% leaf area damaged</td>
</tr>
<tr>
<td>7</td>
<td>Susceptible</td>
<td>26 – 50% leaf area damaged</td>
</tr>
<tr>
<td>9</td>
<td>Highly susceptible</td>
<td>More than 50% leaf area damaged</td>
</tr>
</tbody>
</table>

Results and Discussion

Isolated $C. lindemuthianum$ pathogen was inoculated on potato dextrose agar plate. Microscopic observation of spores revealed asexual fruiting body (Fig. 1). The sub culturing of pathogen was carried out on potato dextrose agar plates and slants (Fig. 2). The spore count was adjusted to $3.4 \times 10^4$ spores/ml and it was inoculated at one month old accessions.

Scoring for disease reaction: All the 40 genotypes were scored for disease reaction after inoculation with anthracnose spore suspension culture at 30 days after sowing.

The accessions were rated as highly resistant, resistant, moderately resistant, moderately susceptible and highly susceptible based on 0-9 scale. The genotypes EC500474, IC328848, EC530819, EC530886, EC500641, IC319370, D-line, L-line and EC398483 were highly resistant, whereas EC304657, IC328655, IC342273, IC520842, EC541908, EC530837, EC559574, IC101264, EC057029, EC530923, EC110172 and EC500226 were resistant. Very few genotypes viz., EC500745, Arka komal, IC319827, EC540797, IC229151 showed moderately resistant, whereas the accessions viz., IC311676, IC329154, EC512812, EC530843, IC319825, IC262749, EC129372, EC531078, EC115962, EC540876, Kanchana, Jwala and IC318852 showed susceptibility to high susceptibility (Table 2).

The disease severity was scored 15 days after inoculation. The disease severity was less in L-line (0.19%), Arka Anoop (0.21%), D-line (0.30%) EC500474 (0.39%), EC530819 (0.27%), EC530886 (0.43%), EC500641 (0.29%), IC319370 (0.27%), IC328848 (0.30%), and EC398483 (0.27%) where as it was higher in susceptible check Kanchana (73.89%), EC115962 (66.11%), EC540876 (73.10%), Jwala (68.80%) and IC318852 (65.0%) indicating the virulence of pathogen culture on resistant and...
susceptible checks. The genotypes EC500474, IC328848, EC5030819, EC5030866, EC5006641, IC319370 and EC398483 showed highly resistance for the *C. lindemuthianum* pathogen under field conditions, along with the confirmed resistant lines D-line and L-line. The genotypes EC115962, EC540876, EC531078 and IC318852 showed high susceptibility for the pathogen along with the susceptible checks Kanchana and Jwala. Garzon et al. (2008) reported that, by inoculating BC1F₁ families and controls with spores of *C. lindemuthianum* at a concentration of 1.2×10⁶ spores/ml in water at 15 days after planting with seedlings in the primary leaf stage, around 93% of the plants in the family showed high level of resistance.

Similar studies was reported by Pathania et al. (2006) that, on inoculation of three days old germinating French bean seedlings of twelve common bean varieties, with a spore concentration of 2.1×10⁶ spores/ml and germination seed dip method revealed that, among forty nine French bean genotypes screened with ten races of *C. lindemuthianum* pathogen, one genotype showed highly resistant, sixteen accessions were susceptible and remaining thirty two genotypes possessed race specific resistance.

**References**


