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# A Technique Used for Testing F3 Progeny of Common Bean (*Phaseolus vulgaris*) Cultivars for Resistance to *Colletotrichum lindemuthianum* (SACC and Magn) Schrib

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**Abstract:** F3 lines of common bean, *Phaseolus vulgaris,* from five parental cultivars; V4141, L746, V6816 , V1864, and V1825 were tested for disease symptoms to 4 isolates (gamma, kappa, iota, and delta) of *Colletotrichum lindemuthianum* (Sacc and Magn.) Schrib. More F3 lines were found resistant in cross 2 (V1864 x V4141), and cross 5 (V1864 x L746) against all the four isolates of *C. lindemuthianum*. Cross 4 (V1825 x V1864) was found the most susceptible against all the four isolates of *C. lindemuthianum* .

**Key words:**

## Introduction

Bean anthracnose is a major disease of common beans *Phaseolus vulgaris* in many parts of the world. It is caused by the fungus *Colletotrichum lindemuthianum* (Sacc and Magn.) Schrib. It is distributed worldwide in cool to moderate, temperature and high humidity or free moisture areas. The disease has caused great economic losses in North America, Africa, Europe, and Asia in susceptible bean cultivars (Zaumeyer and Thomes, 1957). Disease losses can reach 100% when highly contaminated seeds are planted under conditions favourable for disease development.

Barrus (1918) distinguished races of *C. lindemuthianum* on *Phaseolus* beans and recognized alpha and beta races on the basis of differential cultivars. Most cultivars were susceptible to at least one race but some were resistant to both. Race delta was discovered by Andrus and Wade (1942) and epsilon by Blondet (1962). Subsequently, Hubbling (1974) and Kruger *et al.* (1977) isolated races lambda and kappa, respectively. Other races such as iota (produced at Wageningen) do exist but are not yet known to have arisen naturally. Race lambda is the most important race in the UK, which includes resistance to race alpha, beta, gamma, delta, and epsilon. Pathogenicity of the fungus is very unstable and 'Are' gene has become the most important source of resistance in Europe (Mastenbroek, 1960) and other regions of the world. It originates from a Venezuelan line (Cornell 49-242), is easy to incorporate in cultivars and gives complete protection to the races alpha to epsilon and lambda. The other sources of resistance for most of the races of anthracnose were found in Mexico 222, Mexico 227, Ab 136, Kaboon, PI 165.422, and PI 165.426 etc.. The first source of resistance against alpha and beta races were found in 1918-19, and were governed by a single dominant gene.

*Colletotrichum lindemuthianum* is seed-borne disease and causes anthracnose of leaves, stems, and pods. The characteristic symptoms of anthracnose on seed consist of yellow to brown spots where as on leaves reddish spots and lower surface vein dis-colourarstion occurs. The symptoms may become apparent on the upper leaf surface and severe infection may cause leaf tip and edge burning or death of the entire leaf and growing point. Infection of pods produces reddish -brown to black circular spots which develop into light coloured cankers, 3-25 mm in diameter. The lesion on pods are normally surrounded by a dark brown border. In humid conditions a gelatinous pink mass of conidia may cover the center of the lesion. In severe cases the disease causes leaf necrosis, defoliation, and malformation of the pods. Lesions on pods may become pitted and extend to seeds, which then show dark pods (Chaves, 1980).

Various control methods such as application of fungicides (Capotal, Benomyl, and Carbendazim), use of clean seeds, and resistant verities have been used in the past. The present studies were conducted to screen out resistant varieties of *P. vulgaris* from crossing between various parental lines and F3 progenies against *C. lindemuthianum* with reference to the severity of symptoms after inoculation. The five parental cultivars used in this study were from the germ plasm collection of the University of Cambridge and possessed the following characteristics.

**V4141:** Originally from Turkey and was resistant to *P. phaseolicola* of Race 1 (45-2) and Race 2 (45-4) but its nature of resistance was unknown. Plants have indeterminate growth habit and its seeds are mottled off-white and pink in colour.

**L746:** Breeding line from Czechoslovakia. Plant has determinate growth habit, early maturity and white seed in colour.

**V6816:** Harofleet cultivars containing 'Are' gene from Cornell 49-242. Resistant to anthracnose. Plant is bushy type with indeterminate growth and white colour seeds.

**V1864:** Cofinel cultivars from Uganda. It was resistant to anthracnose but the nature of resistance was not known. Plant has determinate growth habit and seed is light brown.

**V1825:** Originally from USA, variety name is US Pinto 114. Resistant to some local races of rust, indeterminate growth habit seed is light brown.

## Materials and Methods

Studies were conducted during 1990 - 91 on common bean (*P. vulgaris)* cultivars for their resistance to anthracnose at the Applied Biology Field Station, University of Cambridge, England. Seeds of F3 generation of 5 crosses and their respective parental varieties were allowed to germinate in 6 cm. Jiffy pots containing laytag beads. All pots were kept in an environment chamber (Conviron Model E15) controlled

at 20 °C. The pots were watered daily with phostrogen solution @ 4.375 g/10 liter of distilled water.

After germination, six plants/ treatment with three replications, were shifted to a glasshouse at 20 °C for inoculation studies and were potted individually. All pots were arranged in a randomized complete block design. The pathogen, *Colletotrichum lindemuthianum* of gamma, kappa, iota, and delta isolates was obtained from Plant Breeding International Cambridge, and sub-cultured on nutrient agar for 24 hours at 20 °C.

As the seedling approached 7-10 cm. length, a 6 mm (diameter) plug of fungal mycelium of four different isolates of *C. lindemuthianum* (gamma, kappa, iota, and delta) were taped between the hypocotyl and cotyledon of intact plant. After inoculation, the plants were covered with plastic bags and were watered by phostrogen solution as needed.

Dark brown lesions appeared on the stem after 7-10 days and later on sometime caused the stem to breakdown by spreading on the stems of susceptible plants (Figure 1). Scoring was made 14 days after inoculation on the basis of disease measurement (mm) on 0-5 scale (Dixon and Doodson, 1973).

0 = No symptom
2 = disease lesions up to 2 mm
3 = disease lesions from 2.1-5 mm
4 = disease lesions from 5.1- 10 mm
5 = > 10.1 mm disease lesion
Resistant = 0 - 7.49 mm, Susceptible = > 7.50

After scoring, resistant or partial resistant plants were shifted to 6 inches pots containing John Inns compost No. 2 as the growing medium (consisting of sterilize loam, peat and sand mixed in the ratio of 7:3:2 and containing 6.12 kg John Inns base fertilizer and 500 grams lime/m3.

Data were analyzed using the analysis of variance technique by Genstat 5 computer program. The mean differences among crosses and lines within crosses were tested by Least Significant Difference test (LSD) at 5% level of probability.

## Results and Discussion

**Parents inoculated by Gamma Isolates:** A significant response to gamma isolates of *C. lindemuthianum* was found between parental verities (Table 1) as indicated by comparing the differences between parental variety means at 5% level of probability. The mean disease measurement (Table 3) indicated that variety V1825 and breeding line L746 were of similar order of magnitude, whereas variety V1864 and V6816 had no response and verity V4141, V1825 and L746 were highly susceptibile to gamma isolates of the pathogen. These results suggest that verities V6816, and V1864 were more resistant than V4141, V1825, and L 746.

**F3 population inoculated by Gamma Isolates:** The analysis of variance (Table 2) of F3 population shows that there were highly significant differences among crosses and lines within crosses in response to gamma isolates of *C. lindemuthianum* following hypocotyl inoculation of F3 intact plants. A significant response was found in lines within cross 1, and cross 5. All other crosses were non-significant, however, the mean disease measurement (mm) in F3 progenies (Table 3) shows no variations between crosses 1, 3, 4 and cross 5, but cross 2 differ significantly from all the four crosses in response to gamma isolate of *C. lindemuthianum*.

Table 1: Analysis of variance of mean disease scores (mm) following hypocotyl noculation of parental verities by Gamma, Kappa, Iota, and Delta isolates of *C. lindemuthianum*

| Source of variation | D.F | M.S | V.R | F.Pr |
|---|---|---|---|---|
| **i. Gamma Isolate:** | | | | |
| Block | 2 | 3.74 | | |
| Parent | 3 | 188.90 | 12.06 | ** |
| Residual | 6(2) | 15.67 | | |
| **ii. Kappa Isolate:** | | | | |
| Block | 2 | 91.27 | | |
| parent | 4 | 340.23 | 3.57 | N.S |
| Residual | 8 | 95.43 | | |
| **iii. Iota Isolate:** | | | | |
| Block | 2 | 15.20 | | |
| parent | 4 | 132.57 | 4.59 | * |
| Residual | 8 | 28.87 | | |
| **iv. Delta Isolate:** | | | | |
| Block | 2 | 6.87 | | |
| parent | 4 | 106.10 | 8.19 | ** |
| Residual | 8 | 12.95 | | |

* Significant at 5% level of probability. ** Significant at 1% level of probability. N.S Non significant.

Table 2: Analysis of variance of mean disease scores (mm) following hypocotyl inoculation of F3 intact plants by Gamma, Kappa, Iota, and Delta isolates of *C. lindemuthianum*

| Source of variation | D.F. | M.S | V.R. | F.Pr |
|---|---|---|---|---|
| **i. Gamma Isolate:** | | | | |
| Block | 2 | 39.62 | | |
| Cross | 4 | 269.68 | 4.07 | ** |
| Lines within cross | 25 | 212.23 | 3.20 | ** |
| Lines within cross 1 | 5 | 236.19 | 3.56 | ** |
| Lines within cross 2 | 5 | 27.22 | 0.41 | N.S |
| Lines within cross 3 | 5 | 108.10 | 1.63 | N.S |
| Lines within cross 4 | 5 | 68.07 | 1.03 | N.S |
| Lines within cross 5 | 5 | 616.80 | 9.30 | ** |
| Residual | 56(2) | 0.6213 | | |
| **ii. Kappa Isolate:** | | | | |
| Block | 2 | 2.75 | | |
| Cross | 4 | 185.50 | 5.99 | ** |
| Lines within cross | 25 | 131.22 | 4.24 | ** |
| Lines within cross 1 | 5 | 79.02 | 2.55 | * |
| Lines within cross 2 | 5 | 66.99 | 2.17 | N.S |
| Lines within cross 3 | 5 | 65.65 | 2.12 | N.S |
| Lines within cross 4 | 5 | 317.43 | 10.26 | ** |
| Lines within cross 5 | 5 | 1232.12 | 3.98 | ** |
| Residual | 57(1) | 0.6213 | | 30.94 |
| **iii. Iota Isolate:** | | | | |
| Block | 2 | 111.89 | | |
| Cross | 4 | 109.89 | 2.15 | N.S |
| Lines within cross | 25 | 220.12 | 4.30 | ** |
| Lines within cross 1 | 5 | 297.20 | 5.80 | ** |
| Lines within cross 2 | 5 | 291.56 | 5.69 | ** |
| Lines within cross 3 | 5 | 121.20 | 2.37 | ** |
| Lines within cross 4 | 5 | 217.40 | 4.24 | ** |
| Lines within cross 5 | 5 | 195.47 | 3.82 | ** |
| Residual | 57(1) | 51.232 | | |
| **iv. Dalta Isolate:** | | | | |
| Block | 2 | 12.97 | | |
| Cross | 4 | 161.24 | 1.86 | N.S |
| Lines within cross | 25 | 228.76 | 2.64 | * |
| Lines within cross 1 | 5 | 79.43 | 0.92 | N.S |
| Lines within cross 2 | 5 | 762.60 | 8.81 | ** |
| Lines within cross 3 | 5 | 142.69 | 1.65 | N.S |
| Lines within cross 4 | 5 | 100.90 | 1.17 | N.S |
| Lines within cross 5 | 5 | 71.12 | 0.82 | N.S |
| Residual | 54(4) | 86.54 | | |

* Significant at 5% level of probability. N.S Non significant.
** Significant at 1% level of probability.

Table 3: Mean disease score (mm) and significant differences* of parental varieties and F3 progeny following hypocotyl inoculation of intact plant by Gamma, Kappa, Iota, and Delta isolate of *C. lindemuthianum*

| Parental varieties | Mean disease scores | F3 cross | Mean disease scores (mm) |
|---|---|---|---|
| **i. Gamma Isolate:** | | | |
| V6816 | 2.30 c | V6816 x V4141 | 11.61 a |
| V4141 | 19.50 a | V4141 x L746 | 5.73 b |
| V1864 | 0.00 c | V1864 x V4141 | 14.80 a |
| V1825 | 12.30 ab | V1825 x V1864 | 14.95 a |
| L746 | 11.30 b | L746 x V4141 | 14.00 a |
| **ii. Kappa Isolate:** | | | |
| V6816 | 6.30 b | V6816 x V4141 | 14.22 ab |
| V4141 | 31.70 a | V4141 x L746 | 9.89 c |
| V1864 | 6.00 b | V1864 x V4141 | 10.94 bc |
| V1825 | 18.00 ab | V1825 x V1864 | 17.83 a |
| L746 | 19.30 ab | L746 x V4141 | 15.06 a |
| **iii. Iota Isolate:** | | | |
| V6816 | 3.30 b | V6816 x V4141 | 13.33 a |
| V4141 | 19.70 a | V1864 x L746 | 16.23 a |
| V1864 | 3.70 b | V1864 x V1825 | 11.00 a |
| V1825 | 9.70 ab | V1825 x V1864 | 15.61 a |
| L746 | 10.70 ab | V1864 x L746 | 11.00 a |
| **iv. Delta Isolate:** | | | |
| V6816 | 3.30 b | V6816 x V4141 | 10.28 a |
| V4141 | 19.70 a | V1864 x V4141 | 15.39 a |
| V1864 | 3.70 b | V1864 x V1825 | 10.39 a |
| V1825 | 9.70 ab | V1825 x V1864 | 15.75 a |
| L746 | 10.70 ab | V1864 x L746 | 9.72 a |

* Any two means in the category sharing the same letter are not significantly different at 5% level of significance by L.S.D test.

Table 4: Mean disease scores and significant differences* of individual segregating lines of each F3 progeny following hypocotyl inoculation of intact plant by Gamma, Kappa, Iota, and Delta isolate of *C. lindemuthianum*

| F3 Cross Seg. line | V6816 x V4141 | V1864 x V4141 | V1864 x V1825 | V1825 x V1864 | V1864 x L746 |
|---|---|---|---|---|---|
| **i. Gamma Isolate:** | | | | | |
| 1 | 26.33 a | 3.33 a | 10.67 a | 12.67 a | 3.67 c |
| 2 | 4.67 b | 10.00 a | 13.67 a | 19.34 a | 8.33 bc |
| 3 | 5.00 b | 4.37 a | 10.67 a | 18.33 a | 6.67 bc |
| 4 | 4.67 b | 7.33 a | 10.00 a | 16.67 a | 17.33 b |
| 5 | 17.67 ab | 2.00 a | 19.00 a | 6.00 a | 41.67 a |
| 6 | 11.33 b | 7.33 a | 25.00 a | 16.67 a | 6.33 bc |
| **ii. Kappa Isolate:** | | | | | |
| 1 | 9.33 c | 11.33 a | 5.67 a | 18.33 b | 15.33 ab |
| 2 | 19.67 ab | 14.02 a | 9.67 a | 30.33 a | 11.67 bc |
| 3 | 9.00 c | 5.00 a | 5.67 a | 27.33 ab | 5.33 c |
| 4 | 20.33 a | 7.33 a | 13.00 a | 19.00 b | 18.67 ab |
| 5 | 11.00 bc | 14.00 a | 16.00 a | 5.33 c | 24.33 a |
| 6 | 16.00 abc | 7.33 a | 15.67 a | 6.67 c | 15.00 b |
| **iii. Iota Isolate:** | | | | | |
| 1 | 1.33 c | 9.67 bc | 5.00 b | 22.33 ab | 16.33 a |
| 2 | 16.33 b | 26.67 a | 18.33 a | 19.67 abc | 3.33 bc |
| 3 | 5.33 bc | 12.00 bc | 11.33 ab | 13.33 bcd | 0.00 c |
| 4 | 14.00 b | 28.00 a | 2.33 b | 25.67 a | 12.67 ab |
| 5 | 13.00 bc | 4.39 c | 12.00 ab | 9.67 cd | 21.67 a |
| 6 | 30.00 a | 16.67 ab | 17.00 a | 3.00 d | 12.00 ab |
| **iv. Delta Isolate:** | | | | | |
| 1 | 7.67 a | 3.00 b | 21.67 a | 17.33 a | 9.00 a |
| 2 | 15.00 a | 47.33 a | 3.67 a | 8.00 a | 8.67 a |
| 3 | 3.00 a | 10.33 b | 10.33 a | 14.33 a | 13.33 a |
| 4 | 12.67 a | 11.33 b | 11.00 a | 23.67 a | 7.33 a |
| 5 | 7.00 a | 9.67 b | 11.67 a | 20.00 a | 17.00 a |
| 6 | 16.33 a | 10.67 b | 4.00 a | 11.17 a | 3.00 a |

* Any two means in the category sharing the same letter are not significantly different at 5% level of significance by L.S.D test.

From the mean disease measurement (mm) in lines within cross 1 and cross 5, three F3 lines were found resistant (score < 7.49 mm) to gamma isolate of *C. lindemuthianum* i.e. 50% F3 lines were resistant (Table 5). In cross 2, out of six F3 inoculated lines, five F3 lines and in cross 4, one F3 line was resistant to gamma isolate of *C. lindemuthianum*. Cross 3 was the only one which showed no resistant plants in the F3 generating population (Table 5).

**Parents Inoculated by Kappa Isolate:** The analysis of variance (Table 1) indicated that there were no significant variation between parents in response to kappa isolate of *C. lindemuthianum*. However, the mean disease measurement (Table 3) showed minor difference between parents at 5% level of probability. The variety V4141 is significantly different from V6816 and V 1864, showing greater susceptibility to kappa isolate followed by L746 and V1825. Variety V6816 and variety V1864 have similar

Table 5: Summary, showing percent of F3 progeny plants in each cross that were resistant against the four races of *Colletotrichum lindemuthianum*

| Races | Cross 1 (V6816 x V4141) | Cross 2 (V1864 x V4141) | Cross 3 (V1864 x V1825) | Cross 4 (V1825 x V1864) | Cross 5 (V1864 x L746) |
|---|---|---|---|---|---|
| Gamma | 50% | 83% | Nil | 17% | 50% |
| Kappa | Nil | 50% | 33% | 33% | 17% |
| Iota | 33% | 17% | 33% | 17% | 33% |
| Delta | 33% | 17% | 33% | Nil | 33% |

Fig. 1: Anthracnose Resistant and Susceptable plants

response for resistance to kappa isolate of *C. lindemuthianum*.

**F3 Population inoculated by Kappa isolate:** The analysis of variance (Table 2) indicated significant difference among crosses and lines within crosses except cross 2 and cross 3. The mean disease measurement (Table 3) showed that cross 1, 4, and cross 5 were not significant from each other but differed significantly from cross 2 in response to kappa isolate of *C. lindemuthianum*. In cross 1 all the six F3 lines were susceptible (Table 4) but within lines they differed significantly in response to kappa isolate of *C. lindemuthianum*. In cross 4, two F3 lines and in cross 5 only one (F3 segregating plant were found resistant in response to kappa isolate of *C. lindemuthianum*. Moreover, in cross 2, 3 F3 lines, and cross 3, two F3 plants in each of these crosses showed resistance to kappa isolate.

**Parents Inoculated by Iota Isolate:** Significant differences (Table 1) were also found among the parents in response to iota isolate of *C. lindemuthianum*. However, the mean disease measurement (Table 3) shows that varieties V6816 and V1864 were significantly different from variety V4141 but not from V1825, and L746. Variety V6816 and V1864 were found resistant in response to iota isolate of *C. lindemuthianum*, containing Are gene from Cornell 49-242, but V1864 has unknown sources to some isolates of *C. lindemuthianum*.

**F3 population Inoculated by Iota Isolate:** Table 2 shows that crosses were not significantly different in response to iota isolate of *C. lindemuthianum* but variation among lines within crosses and individual crosses was significant. The mean disease measurement in all crosses (Table 3) shows similar order of magnitude in response to susceptibility of iota isolate of *C. lindemuthianum*. However, the mean disease value within individual crosses (Table 4) shows variability in response to resistance and susceptibility. In cross 1, 3, and cross 5, two F3 plants, in cross 2 and cross 4, only one F3 plant in total of six F3 plants were resistant to iota isolate of *C. lindemuthianum*. (Store < 7.49)

**Parents Inoculated by Delta Isolate:** The analysis of variance (Table 1) shows that there were significant differences among all the parents. As indicated in Table 3, V1864 and V1816 have similar response of resistance to delta isolate of *C. lindemuthianum*. The mean disease values indicated that V6816 and V1864 were more resistant and V4141 more susceptibility than any other verities tested.

**F3 population Inoculated by Delta Isolate:** Non significant difference was found among crosses and lines within individual crosses except lines within cross 2 and lines within crosses (Table 2). The mean disease measurement (Table 3) indicated that all the crosses have similar order of magnitude in response to delta isolate of *C. lindemuthianum* and were found susceptible. However, the mean disease values within crosses 1, 3, and cross 5 have two F3 lines and cross 2 have only one resistant plants out of six F3 inoculated lines. Cross 4 had all six F3 susceptible lines in response to delta isolate of *C. lindemuthianum* (Table 4). From the summary (Table 5) it is clear that cross 4 was the most susceptible cross against all the four isolates of *C. lindemuthianum* .

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