

Original Research Article

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## To Study HCN Production by French bean (*Phaseolus vulgaris*) Accessions and Their Potential to Resistance against Anthracnose

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### ABSTRACT

#### Keywords

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Frenchbean, *Phaseolus vulgaris* L. ( $2n = 2x = 22$ ) is one of the most important leguminous vegetable grown for its tender fleshy green pods, shelled green seeds, and also dry beans. Cyanogenesis has been one of the important factors of plant defense mechanism. Many plants liberate HCN after mechanical or chemical injury to their cells or in course of fungal infection. The objective of current study was to correlate between the disease resistance against Anthracnose and HCN production in Frenchbean. The study was based on method of HCN estimation using picrate strips and their quantitative estimation through Cyanogen Kit Colour Chart (ppm) and by estimating the absorbance of picrate solution and Standard Curve of absorbance against HCN concentration ( $\mu\text{g/ml}$ ). According to the results the highest HCN content has been seen in lines L line and D line.

### Introduction

Frenchbean, *Phaseolus vulgaris* L. ( $2n = 2x = 22$ ) also known as Snapbean, Kidney Bean, Garden Bean, or String Bean, is one of the most important leguminous vegetable grown for its tender fleshy green pods, shelled green seeds, and also dry beans (Praveen Kumar Angadi *et al.*). It has anti diabetic property and is good for natural cure of bladder burns and cardiac problems, diarrhoea, sciatica and tenesmus. It is nutritive vegetable rich in protein (1.7g), calcium (132 mg), Thiamine (0.08 mg), and vitamin C (24 mg for 100 grams of edible pods). French Bean is

originated from Central America and Peruvian Andes in South America. It spread to Europe during 16th and 17th centuries and reached England by 1594. It was introduced in India during century from Europe. The statistics with respect to this crop is very deficient owing to the small area of production and short duration. However as per FAO estimates it is grown in world in an area of 0.83 m ha with annual production of 5.64 m t with productivity of 6.76 tons per hectare. In India French bean is mainly grown in Himachal Pradesh, Punjab, Haryana, Uttar Pradesh, Gujarat, Madhya Pradesh, Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu.

French Bean is grown in an area of 0.15 m ha with annual production of 0.42 m t and productivity of 2.8 t per ha (FAO STAT, 2002).

French bean is susceptible to many fungal and bacterial diseases like Anthracnose, Powdery mildew, Bacterial brown spot, Bacterial wilt. But several resistant lines of Frenchbean are capable producing HCN, which acts as a defence mechanism against different kinds of diseases. Anthracnose is one of the commonly occurring disease, in this experiment, An afford was made to correlate between the disease resistance against Anthracnose and HCN production in French Bean.

Anthracnose is mainly a seed borne disease caused by a fungus which has a wide host range on many legume species. It is caused by *Colletotrichum lindemuthianum*. The disease can cause a serious loss in bean crops in temperate and subtropical zones. Leaves, stems, and pods of bean plants are susceptible to infection. Small reddish brown, slightly sunken spots form on the pods and rapidly develop into large, dark sunken lesions. In moist weather, masses of pink spores develop on lesions. Black sunken spots, similar to those on pods are developed on stems and leaf stalks. Infection of leaves cause blackening along the veins, particularly on the under surface (Elizabeth Minchinton, Knoxfield 1999). The fungus can survive on contaminated seed and on crop debris for at least two years. Development of the disease is most rapid in warm, damp conditions. Symptoms appear between 18 and 25°C but are delayed or prevented by temperatures outside the range of 7-33 C (Elizabeth Minchinton, Knoxfield, 1999).

Cyanogenesis is ability of plants to release hydrogen cyanide, has been known for several centuries. Since its first description in plants in 1803, the phenomenon of Cyanogenesis was

recognised in 3000 species of higher plants distributed throughout 110 different families of ferns, gymnosperms, and both monocots and dicots (Conn, 1981). However, only in approximately 300 plant species has the source of HCN has been identified. In certain sapindaceous seeds HCN may arise cyanolipid hydrolysis. More frequently HCN production in higher plants results from the catabolism cyanogen glycosides. All higher plants probably low levels of HCN as a co-product of ethylene biosynthesis (Kende, 1989). Many economically important food plants are highly capable of acute cyanide poisoning of animals including man. Additionally in areas of the world where cassava and lima beans comprise major items of diet, chronic cyanide poisoning and associated pathological conditions still exist (Poulton, 1989).

Weakly cyanogenic plants liberate only small amounts of HCN against which their detoxification reaction provide sufficient protection. However, in strongly cyanogenic plants the liberation of HCN is rapid and the resulting HCN concentration in the leaves is too large to be detoxified immediately by plants. Consequently the plant defence responses will be hampered. The susceptible, highly cyanogenic plants possess the same genetic potential for the defence reactions as weakly cyanogenic plants but are inhibited by HCN produced endogenously in course of tissue colonization.

The anthracnose of common bean was first identified in the fruit and vegetable garden of the agriculture institute of Popplesdorf Germany by Lindemuth. By 1878, Saccardo and Magnus had made many observations on the cause of anthracnose disease, recording their results in *Michelia*. They concluded that it was caused by a fungus, which they named *Gloeosporium lindemuthianum* after Lindemuth himself. Several years later, Briosi and Cavara discovered the presence of setae

on the fungus, reclassifying it from *Gloeosporium* to *Colletotrichum*.

## **Materials and Methods**

### **Culture of Anthracnose**

Anthracnose culture was developed from the infested leaves of French bean in lab. Diseases isolates have been collected from experimental fields. Isolated plant parts have been cut into small bits and surface sterilized with 0.1 percent mercuric chloride (HgCl<sub>2</sub>) solution for about one minute and washed repeatedly thrice in sterile distilled water before transferring them to sterile petriplates containing PDA media (5mm) under LF (laminar air flow chamber). Excess water has been wiped off with sterile tissue paper. Petriplates containing diseased plant parts were kept in a room temperature for seven to ten days under aseptic conditions and observed at regular intervals for growth of the pathogen. After the incubation period, fungal pathogens were observed under compound microscope. For purification of the cultures, single spore isolation technique was followed pure culture was prepared for getting species purity. Subcultures were prepared in media slants for conducting experiments without disturbing pure culture. Slants of pure cultures have been kept in refrigerator at 5 degrees Celsius for storage. Sub culturing was done subsequently at an interval of 30 days in order to maintain virulence of culture purpose. Three weeks old plants were inoculated with spore suspension (10<sup>6</sup> conidia/ml) of pathogen *C. lindemuthianum* to check for resistance against *C. lindemuthianum*.

Total seven different types of French Bean were taken for the estimation, out of which six were collected from Garhwal region of Uttarakhand, they are L line, D line, GFB 24, GFB 22, GFB 30. The remaining two Falguni, Mridula which are common varieties that are

under cultivation. The disease resistance of four lines L line, D line, and GFB 24, GFB 30 is known and line GFB 22, is susceptible line. The disease resistance of remaining two Falguni, Mridula is unknown. Two replicates of each line were taken. The seeds are sown in the pots of medium size which are filled with soil. The soil should be mixed with the farm yard manure to prevent the malnutrition of the plants. Five seeds of each line are sown in the three pots. The seeds are sown at a depth of 5cm (2inches) for better and faster germination. The seeds should be watered immediately after sowing, the pots should not be over flooded to prevent rotting and optimum amount of water should be given to seeds for better germination. The pots are placed where sunlight is abundant and they should be watered alternate days. The seeds are germinated within one week, after the seedlings become one month older they are grown under controlled conditions (Fig. 1 and 2).

### **Method of HCN Determination**

The method of HCN estimation was essentially derived from that of Nowosad and Mc Vicar. The procedure was as follows: The above method was altered with slight modifications according to requirements and resources available. The Whatman filter paper is cut into strips of 1.5 cm wide and 5 cm in length for making picrate strips. After this the 100 ml picrate solution is made by dissolving 2.5 grams of sodium carbonate and 0.5 grams of picric acid in 100 ml of distilled water. The solution is mixed thoroughly until the sodium carbonate and picric acid. A clear yellow coloured solution is obtained after thorough mixing. The solution is kept in dark or shady conditions until it is used. The strips of filter paper are now dipped in the picrate solution, taken out and kept aside in dark to dry them slightly. The test tube with closed lids is used in this method instead of vials. The chloroform is taken into a beaker and is closed

with aluminium foil to prevent it from evaporation. The whole equipment is kept in box in shade and taken directly to the field where the pots of French bean are present. The leaves of medium size from each replicate of each line are taken and placed in the test tube. A drop of chloroform of 0.5 ml is dropped on the leaf with help of micro pipette. The pre-prepared picric acid strips are placed above leaf without touching the leaf, the whole procedure should be carried out inside box in shady conditions. The test tubes with leaves and picric acid strips are immediately taken to seed germinator, placed in dark and at a temperature of 25 for 48 hours. After 48 hours the picric acid strips are checked for the reddish colour which is proportional to the HCN production.

#### **Semi quantitative determination of cyanide using picrate paper**

Following the qualitative assay, the picrate papers were soaked in 5ml of distilled water for 3 minutes to prepare picrate solution. Absorbance of picrate solution was read at 510 nm using U.V. visible spectrophotometer. The linear calibration curve of cyanide used to determine HCN content in French Bean sample was as drawn in figure 3.

#### **Qualitative determination of cyanide using picrate paper**

The colour of picrate papers matched against the colour chart shown in the figure 4 for the qualitative determination of Cyanide in ppm. This procedure was repeated for the blank (same procedure was used with no French bean/ HCN put in the bottle) and the standard HCN solution.

#### **Data analysis**

The obtained data tabulated and expressed as standard error of the mean. The student T test

was employed to determine statistical significance of obtained results (Table 1–3).

#### **Ethical considerations**

All reagents and chemicals were handled and disposed of according to the set laboratory regulations. Good laboratory practice was upheld to ensure safety.

#### **Results and Discussion**

##### **Estimated cyanide content in parts per million from colour chart**

The lines L Line, D Line, had shown HCN concentration of 10 ppm before infection and after infection 65 ppm and 60 ppm respectively. The varieties falguni and Mridula had shown HCN content of 12 ppm, 15 ppm before infection and 50 ppm, 30 ppm after infection respectively. The lines GFB 22, GFB 30 had shown HCN content of 5 ppm before infection and after infection 5 ppm and 50 ppm.

The Line GFB 24 has shown a HCN concentration of 10 ppm before infection and 50 ppm after infection. The results are given in Table 2.

##### **Determined cyanide content from the picrate paper**

Spectrophotometric determination of the picrate papers demonstrated that the disease resistant lines Line L, Line D, GFB 24, and varieties Falguni, Mridula demonstrated cyanide concentrations of 0.13µg/ml before infection and 0.15, 0.15, 0.14, 0.14, 0.13µg/ml after infection respectively. The Line 22 has shown cyanide production of 0.12µg/ml before infection and 0.12µg/ml after infection. The line GFB 30 has shown a HCN content of 0.12µg/ml before infection and 0.14µg/ml after infection (Table 3).

**Table.1** Values for standard calibration curve Preparation

Line	Absorbance	
	Before Infection	After Infection
L line	0.160	0.286
D line	0.160	0.281
Falguni	0.161	0.250
Mridula	0.163	0.230
GFB 24	0.160	0.251
GFB 22	0.150	0.157
GFB 30	0.150	0.250

**Table.2** Values of HCN content obtained using colour chart

Line	Concentration in ppm		t calculated
	Before Infection	After Infection	
L line	10 ppm	65 ppm	
D line	10 ppm	60 ppm	
falguni	12 ppm	50 ppm	4.41
Mridula	15 ppm	30 ppm	
GFB 24	10 ppm	50 ppm	
GFB22	5 ppm	5 ppm	
GFB 30	5 ppm	50 ppm	

**Table.3** Values of concentration of HCN obtained using spectrophotometer

Line	Concentration µg/ml		t calculated
	Before Infection	After Infection	
L line	0.13	0.15	
D line	0.13	0.15	
falguni	0.13	0.14	2.57
Mridula	0.13	0.13	
GFB 24	0.13	0.14	
GFB 22	0.12	0.12	
GFB 30	0.12	0.14	

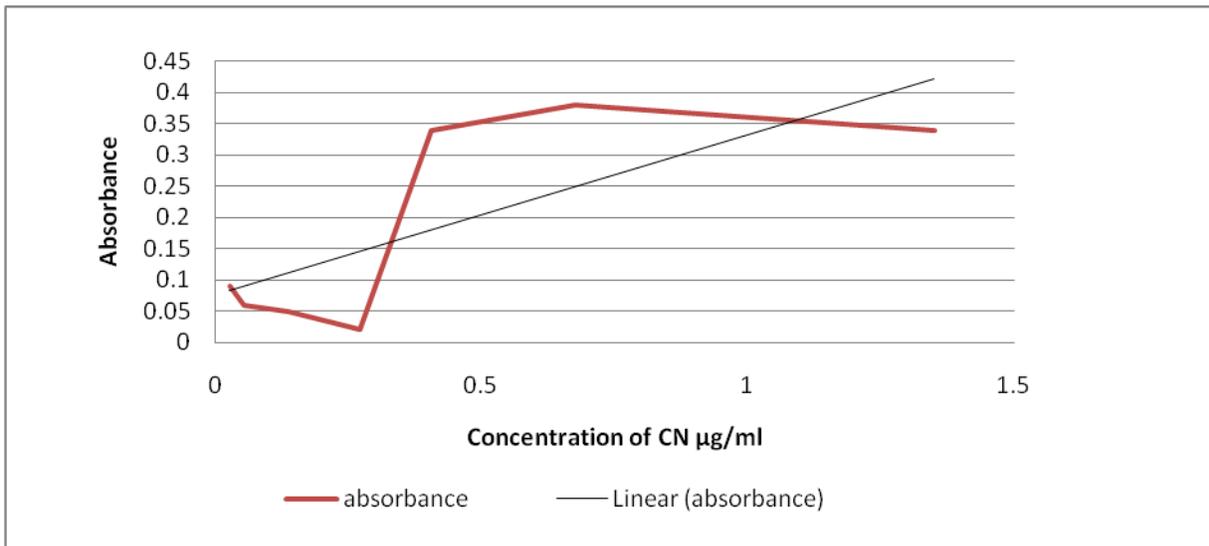
**Fig.1** Culture of Anthracnose



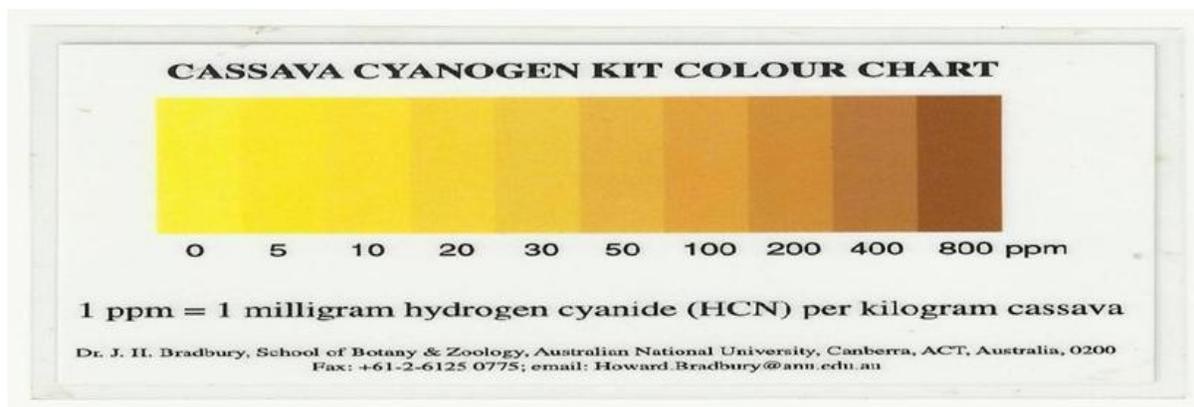
**Fig.2** French bean leaf infected with anthracnose



**Fig.3** A standard curve of absorbance against HCN concentration



**Fig.4** Cyanogen kit colour chart



**Fig.4** One month old plant of different French bean accessions before infection of *Colletotrichum lindemuthianum* (Anthracnose)



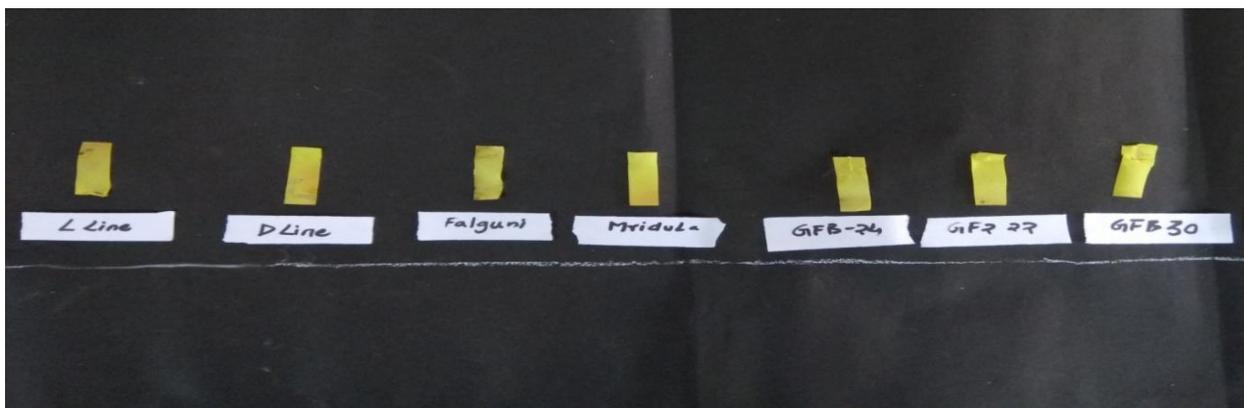
**Fig.5** Effect of anthracnose fungus on French bean plants after 10 days of artificial inoculation of anthracnose culture



One month old plants were inoculated with the culture of anthracnose. French bean accession 24 and 30 were resistant to anthracnose in comparison to other accessions and varieties

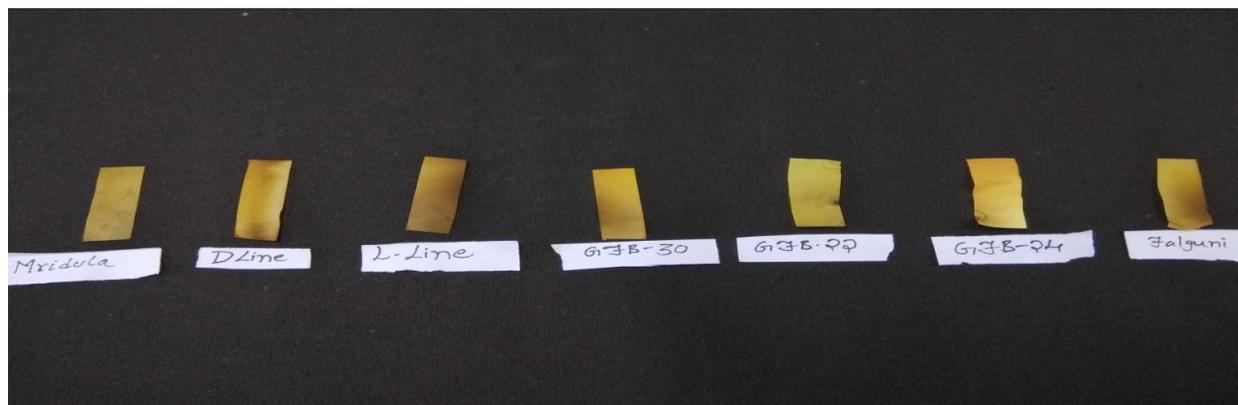


**Fig.6** HCN production by different French bean accessions and varieties before inoculation of anthracnose



Mridula and Falguni- Cultivars, L- Line and D-line- Resistant line for anthracnose, GFB- 22, GFB- 24, GFB-30- French bean accessions collected from Garhwal region.

**Fig.7** HCN production by different French bean accessions and varieties after one week of inoculation of anthracnose



Mridula and Falguni- Cultivars, L- Line and D-line- Resistant line for anthracnose, GFB- 22, GFB- 24, GFB-30- French bean accessions collected from Garhwal region.

By the above values which were obtained through different methods of HCN estimation, it can be clearly stated that the disease resistance of lines L line, D line, GFB 24, GFB 30, and the cultivars Falguni and Mridula against Anthracnose can be directly related to HCN production of the given accessions. As the disease resistant lines L Line, D line had shown a highest production HCN content after infection they can be considered as highly resistant to the Anthracnose disease. Whereas remaining accessions GFB 30, GFB 24, Falguni and Mridula had also shown considerable amount of HCN production. So, their disease resistance can be correlated to their HCN production. The susceptible line GFB 22 has shown least amount of HCN content before and after infection which can be clearly mentioned as reason for its susceptibility for Anthracnose disease.

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