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Screening of *phaseolus vulgaris* Cultivars Growing in Various Areas of Jammu and Kashmir for Anthracnose Resistance

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Abstract- *Phaseolus vulgaris* is an economically important crop. The most important disease which hampers its production is anthracnose which is found in almost every bean growing region of the world. Anthracnose in beans is caused by a hemibiotrophic fungus, namely *Colletotrichum lindemuthianum.* Use of resistant genotypes will provide a long lasting solution to the economic losses caused by anthracnose as this method is biologically safe as well as cost effective. For the present investigation, nearly ten *C. lindemuthianum* isolates collected from Baramulla, Kashmir were used. Inoculum of each isolate was produced by inoculating bean extract medium with a monosporic culture of *C. lindemuthianum*, incubated at 25ºC for seven days, the spore suspension culture filtered through double layered muslin cloth and inoculum load was adjusted to 1.2 X 10^6 spores per milliliter with the help of a haemocytometer. Seed dip method was used for the inoculation of the test plant. The inoculated seeds were sown in sand and were kept at 25ºC for the period of one month. The disease reaction was scored after twelve days of inoculation following 0-5 scale where 0 = no disease; 1 = pin point lesion; 2 = small lesion; 4 = large deep lesion and 5 = sunken lesions with rotten apex. Two broad categories of the accessions emerged; infected (infection rate high or mild) or non-infected. Based on the presence or absence of infected plants accessions were labeled as susceptible or resistant.

I. INTRODUCTION

Among grain crops, pulses (food legumes) rank third after cereals and oilseeds in terms of total world production. Pulses are rich in proteins and represent an important source of dietary protein for humans and animals. The proteins are generally composed of high amount of lysine, while the amount of methionine and cysteine is less. However, consumption of legumes and cereals results in a balanced diet of energy and protein. Legumes are also an important source of some essential minerals [(1)]. The legumes have been observed to reduce blood cholesterol levels [(2)]. Among major food legumes, common bean (*Phaseolus vulgaris* L.) is third in importance, has broadest genetic base and is grown and consumed in almost every part of the world [(3,4)] . Common bean (*Phaseolus vulgaris* L.) is important food grain legume crop, cultivated in almost every part of the world. This species is a diploid with 2n=2X=22, it is predominantly a self crossing species with only exception of about 3%. [(5)]

Although, beans are cultivated throughout India, Himalayas hold the richest diversity of common bean. Common bean production is influenced by both biotic and abiotic stresses; biotic factors are responsible for major losses. Six major diseases (anthracnose, rust, angular leaf spot, common bacterial blight, Bean Golden Mosaic Virus and Bean Common Mosaic Virus) are known to hamper common bean production. However, the most important among these is anthracnose which is found in almost every bean growing region of the world [(6)]. It is considered as one of the most destructive disease [(7)]. Anthracnose is caused by a hemibiotrophic fungus, namely *Colletotrichum lindemuthianum.* This disease is known to cause total loss of the crop. Disease generally occurs by contaminated seeds or infected plant debris [(8)]. This disease may lead to major or total crop loss, particularly in case a susceptible variety is sown [(9,10)]. The anthracnose genetics has been studied for a long time [(11)]. This host-pathogen interaction was the first report of race-cultivar specificity [(12)]. The chemical control method has been used to check the disease, however, it is very expensive and causes severe threat to the health and environment. Use of resistant genotypes will provide a long lasting solution, as this method is biologically safe as well as cost effective. Therefore the present study was aimed to characterize the Common bean accessions growing in J&K state for resistance and susceptibility.

II. METHODOLOGY

For the present study forty four common bean accessions growing in various areas of Jammu and Kashmir were collected. Screening for disease resistance in these accessions was achieved by challenging the plants with the pathogen. The inoculums of *Colletotricum lindemuthianum* were applied to all the accessions and disease incidence and infection rate were determined by visual rating. The plants were classified as susceptible and resistant. Infected pods of various accessions of beans were collected from different areas of Baramulla district in Kashmir valley at regular time intervals. After collection, the infected pods were placed in between the folds of newspapers for drying. Efforts were made to remove as much moisture as possible, in order to avoid cross contamination by other fungi. The samples were kept in the paper bags marked with different accession

numbers. The bags were wrapped in polythene and kept at 4ºC to minimize the degradation and to prevent the contamination. Potato dextrose agar medium (PDA), the basic medium for culturing of many fungi, was successfully used for *Colletotrichum* isolates collected from various bean accessions. The antibiotic, chloramphenicol (50µg/ml) was added to the medium to avoid bacterial contamination. In addition bean pod extract medium was also used. Infected pods were washed with distilled water, treated with disinfectant to eliminate the bacterial and other contaminants from the pod surface. Small pieces of the infected pods were inoculated on PDA and kept at 25ºC. The fungus started growing after a week of inoculation and thereafter, its growth was monitored every week. The fungus isolated from infected pods was again sub-cultured on PDA to obtain the pure cultures. For preparing a pure culture, the spores were picked from the petriplates and sub-cultured on fresh PDA plate until pure culture was obtained. Using this technique five isolates of *C. lindemuthianum* were obtained. Pure cultures of *C. lindemuthianum* were stored at 4ºC. These were maintained as mother cultures for further study. For long term storage, glycerol cultures were raised. Spores were mixed in 70% glycerol in storage vials and were stored at -80ºC. In addition to PDA medium, bean extract medium was also used for the sporulation of the fungus. For preparation of bean extract medium, young bean pods were surface sterilized and boiled in water. The extract was then filtered through double layered muslin cloth, and to the filtrate 2% dextrose was added. The pH of the medium was adjusted to 7.0 and 2% agar was added prior to autoclaving. For the present investigation, three *C. lindemuthianum* isolates collected from Baramulla, Kashmir were used. Inoculum of each isolate was produced by inoculating bean extract medium with a monosporic culture of *C. lindemuthianum*. After inoculation, the plates were incubated at 25ºC for seven days. The surface of the culture was scraped with a sterilized glass rod, and the spore suspension was prepared in sterilized water from seven day old sporulating cultures of all the three isolates. The spore suspension culture was filtered through double layered muslin cloth and inoculum load was adjusted to 1.2 X 10^6 spores per milliliter with the help of a haemocytometer. Seed dip method was used for the inoculation of the test plant. Five surface sterilized seeds of each accession were germinated on sterilized filter paper under aseptic conditions. The seeds were surface sterilized by dipping in 70% ethanol for 5min, followed by treatment with 0.1% mercuric chloride for 10min and finally 2X wash with double distilled water. Five day old germinating seeds of each accession (5 seeds) were dip inoculated in the spore suspension for 5min after removal of the seed coat. The inoculated seeds were sown in sand and were kept at 25ºC for one month. The disease reaction was scored after twelve days of inoculation following 0-5 scale [13], where 0 = no disease; 1 = pin point lesion; 2 = small lesion; 3 & 4 = large deep lesion and 5 = sunken lesions with rotten apex.

III. Results and Discussion

The present study being first of its kind, for the screening of resistant common bean accessions growing in Jammu and Kashmir state, is based upon the forty seven common bean accessions collected from various areas (Table 1). During present study the common bean accessions were challenged with *C. lindemuthianum* cultures to screen the resistant and susceptible cultivars. The isolates of *C. lindemuthianum* were collected from infected pods of different cultivars growing in various areas of Kashmir valley. A total of ten fungal isolates were cultured and maintained while in the present study the results with one *C. lindemuthianum* isolate on common bean accessions has been given. Based on the response, the plants were classified as susceptible and resistant (Table 2). This method of screening the bean germplasm for disease resistance is an established one and has been used by many workers including [14]. The latter workers reported that after one week of treatment, the susceptible cultivars showed severe infection on the cotyledons and the hypocotyls resulting in death. Although, slightly susceptible cultivars survived, however, these developed distinct stem and leaf necrosis (Fig 1, 2). On the other hand the resistant cultivars did not show any symptoms of the disease (Fig. 3, 4). Similar results were obtained in the present investigation. The susceptible cultivars showed formation of large lesions with sunken cankers on cotyledons and dead apex, the mild infected plants showed slight infection on cotyledons, while resistant plants were healthy and without any disease symptoms. This method of screening varieties has been used by many other workers in common bean and found to be the most suitable one [15-17]. Therefore, the present study, being the first of its kind to scan the bean germplasm for resistance to anthracnose from various areas of Kashmir region, these resistant varieties can be used for the development of better varieties of beans.

IV. Conclusion

From the present study we concluded that the beans germplasm growing in various areas of Jammu and Kashmir hold the richest repository of anthracnose resistant cultivars. These cultivars can be studied in detail to isolate the resistant genes present in them and can be used for the breeding studies so as to develop a cultivar which can withstand the threat of this deadly disease.

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Table 1: Bean germplasm and its characterization

S. No.	Accession Number	Colour of Seeds	Shape of Seeds	Type of Seeds
1	JUBG50	Pink	Small	Snap
2	JUBG51	Pink	Small	Dry
3	JUBG52	Mottled	Small	Snap
4	JUBG53	Red	Small	Dry
5	JUBG54	Black	Small	Dry
6	JUBG55	Mottled	Big	Dry
7	JUBG56	Brown	Big	Snap
8	JUBG57	Mottled	Big	Dry
9	JUBG58	Red	Big	Snap
10	JUBG59	Brown	Small	Dry
11	JUBG60	Mottled	Small	Snap
12	JUBG61	Mottled	Small	Dry
13	JUBG62	Brown	Big	Snap

14	JUBG63	Brown	Big	Snap
15	JUBG64	White	Small	Snap
16	JUBG65	Light brown	Medium	Dry
17	JUBG66	White	Small	Dry
18	JUBG67	Pink	Medium	Dry
19	JUBG68	Pink	Big	Snap
20	JUBG69	White	Medium	Dry
21	JUBG70	Red	Small	Dry
22	JUBG71	Blackish brown	Small	Snap
23	JUBG72	Mottled	Big	Snap
24	JUBG73	Pink	Small	Dry
25	JUBG74	Pink	Small	Snap
26	JUBG75	Red	Big	Dry
27	JUBG76	White	Small	Dry
28	JUBG77	Mottled	Medium	Dry
29	JUBG78	Mottled	Small	Dry
30	JUBG79	Pink	Big	Dry
31	JUBG80	Pink	Small	Snap
32	JUBG81	Brown	Big	Dry
33	JUBG49	Mottled	Big	Dry
34	JUBG82	Red	Medium	Dry
35	JUBG83	Mottled	Medium	Dry
36	JUBG18	Red	Small	Dry
37	JUBG84	Red	Small	Dry
38	JUBG85	Red	Small	Snap
39	JUBG86	Brown	Big	Snap
40	JUBG87	Brown	Big	Snap
41	JUBG88	Mottled	Medium	Snap

42	JUBG89	Mottled	Big	Snap
43	JUBG90	Red	Medium	Snap
44	JUBG92	Red	Medium	Snap

Table 2: Response of different bean accessions to *C. lindemuthianum*

S. No	Accession	Observations after 1st week	Observations after 2nd week	Observations after 3rd week	resistant	Susceptible
1	JUBG18	Infection on cotyledons, no germination	Highly infected cotyledons, slow growth rate	Shoot apex dead, infection on stem and lower side of leaves	-	+
2	JUBG49	Infection on cotyledons, very slow germination	Highly infected cotyledons, slow growth rate	Infection on stem and lower sides of leaves	-	+
3	JUBG50	Infection on cotyledons, very slow germination	Highly infected cotyledons, slow growth rate	Infection on stem and leaves. Plants very slow growing	-	+
4	JUBG51	Slight infection on cotyledons, fast germination	Healthy plants, growth rate high	Healthy plants	+	-
5	JUBG52	Highly infected cotyledons	No germination	No plant formation	-	+
6	JUBG53	Highly infected cotyledons	Germination very slow	Shoot apex dead	-	+
7	JUBG54	Slight infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
8	JUBG55	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
9	JUBG56	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
10	JUBG57	Slight infection on cotyledons, moderate germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
11	JUBG58	Slight infection on cotyledons germination rate good	Infection on cotyledons, growth rate moderate	No infection on stem and leaves	+	-
12	JUBG59	No infection on cotyledons, fast	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease	+	-

		germination		symptoms		
13	JUBG60	Slight infection on cotyledons, moderate germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
14	JUBG61	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast leaves and stem devoid of any disease symptoms	+	-
15	JUBG62	Slight infection on cotyledons, moderate germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
16	JUBG63	Slight infection on cotyledons, moderate germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
16	JUBG63	Slight infection on cotyledons, moderate germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
17	JUBG64	Highly infected cotyledons	Germination very slow	Shoot apex dead	-	+
18	JUBG65	Slight infection on cotyledons, moderate germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
19	JUBG66	Slight infection on cotyledons germination rate good	Infection on cotyledons, growth rate moderate	No infection on stem and leaves, but first two leaves curled.	+	-
20	JUBG67	No infection on cotyledons, fast germination.	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
21	JUBG68	Highly infected cotyledons, no germination	Germination very slow	Shoot apex dead, infection on stem and lower side of leaves	-	+
22	JUBG69	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
23	JUBG70	Slight infection on cotyledons germination rate good	Infection on cotyledons, growth rate moderate	Healthy plants	+	-
24	JUBG71	Highly infected cotyledons, no germination	Germination very slow.	Shoot apex dead, infection on stem and lower side of leaves	-	+
25	JUBG72	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-

26	JUBG73	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
27	JUBG74	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
28	JUBG75	Infection on cotyledons, no germination	Highly infected cotyledons, slow growth rate	Shoot apex dead, infection on stem and lower side of leaves	-	+
29	JUBG76	Highly infected cotyledons	Germination very slow	Shoot apex dead, infection on stem and lower side of leaves	-	+
30	JUBG77	Slight infection on cotyledons, seed germinated	Infection on cotyledons, plant formation	Healthy plants.	+	-
31	JUBG78	Slight infection on cotyledons germination rate good	Infection on cotyledons, growth rate moderate	Healthy plants	+	-
32	JUBG79	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
33	JUBG80	Highly infected cotyledons	Germination very slow	Shoot apex dead	-	+
34	JUBG81	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptom.	+	-
35	JUBG82	Slight infection on cotyledons germination rate good	Infection on cotyledons, growth rate moderate	Healthy plants	+	-
36	JUBG83	Slight infection on cotyledons germination rate good	Infection on cotyledons, growth rate moderate	Healthy plants	+	-
37	JUBG84	Highly infected cotyledons, no germination	Germination very slow	Shoot apex dead, infection on stem and lower side of leaves	-	+
38	JUBG85	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
39	JUBG86	Slight infection on cotyledons, germination rate moderate	Cotyledons slightly infected, growth rate moderate	Plant healthy with no symptoms of infection on leaves and stem	+	-
40	JUBG87	Highly infected cotyledons, no germination	Germination very slow	Shoot apex dead, infection on stem and lower side of leaves	-	+

41	JUBG88	Slight infection on cotyledons germination rate good	Infection on cotyledons, growth rate moderate	Healthy plants	-	+
42	JUBG89	Slight infection on cotyledons germination rate good	Infection on cotyledons, growth rate fast	Healthy plants	+	-
43	JUBG90	Slight infection on cotyledons, moderate germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-
44	JUBG92	No infection on cotyledons, fast germination	Healthy plant formation	Growth rate fast, leaves and stem devoid of any disease symptoms	+	-