Transfer of resistance to floury leaf spot and bacterial blight in french bean from alien germplasm

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ABSTRACT: Resistance to floury leaf spot caused by Mycovellosiella phaseoli and bacterial (fuscous) blight caused by Xanthomonas axonopodis pv. phaseoli of french bean (Phaseolus vulgaris) was found in alien Phaseolus species P. coccineus and P. acutifolius. An attempt was made to transfer resistance from these species to french bean by means of interspecific hybridization. Two hybrids of P. vulgaris (contender) x P. acutifolius (G 40015 and G 40020) and five of P. vulgaris x P. coccineus (Pc1 and Pc2) were free of floury leaf spot but exhibited varying number of fuscous blight lesions. However, lesion size in these hybrids was highly restricted except in hybrids with G 40015 and G 40020. Five inter-specific hybrids of P. vulgaris (Him1) x P. coccineus were resistant to both bacterial blight and floury leaf spot.

Key words: French bean, scarlet runner bean, tepary bean, Xanthomonas axonopodis pv. phaseoli, Mycovellosiella phaseoli, transfer of resistance, alien germplasm

French bean (Phaseolus vulgaris) also known as common bean, dry bean or kidney bean occupies a premier place among edible legumes in a number of bean growing countries of the world including India. During humid and warm conditions, a wide range of diseases some of which are of widespread occurrence cause significant reduction in pod yield and grain quality. Some of the destructive diseases of french bean are anthracnose (Colletotrichum lindemuthianum), angular leaf spot (Phaeoisariopsis griseola), floury leaf spot (Mycovellosiella phaseoli) and bacterial blight (Xanthomonas axonopodis pv. phaseoli). Resistance in a wide array of germplasm has been found worldwide against anthracnose and angular leaf spot but high level of resistance to floury leaf spot and bacterial blight has not been found.

Floury leaf spot is recurrent in all major bean growing areas of India especially in north–western Himalayan region and results in large scale losses in terms of grain yield due to extensive defoliation (Dev, 1990). Bacterial blight continues to be a major constraint in high rainfall areas. Sources of resistance to X. axonopodis pv. phaseoli have been reported in tepary bean, P. acutifolius (Drijfhout and Blok, 1987; Schuster et al., 1983), runner bean, P. coccineus (Park and Dhanvantary, 1987) and common bean, P. vulgaris (Pompeau and Crowder, 1972; Rava et al., 1987). Schuster et al., (1983) reported that leaves and pods of P. acutifolius lines showed high levels of resistance with no visual symptoms of common blight on leaves. The cross between P. vulgaris and P. coccineus is successful only if the former is used as female parent (Coyne, 1964; Drijfout and Blok, 1987). Since high level of resistance has not been found to both the pathogens in french bean germplasm, an effort has been made to transfer resistance from P. acutifolius and P. coccineus to P. vulgaris against these pathogens.

MATERIALS AND METHODS

Isolation and pathogenicity studies of Mycovellosiella phaseoli

Diseased samples of french bean showing symptoms of floury spot on the under surface of leaves were collected from the University farm. The
pathogen was isolated from infected leaves under aseptic conditions on 2% water agar medium. The culture was maintained in BOD incubator at 25 ± 1°C for 24 h and purified by serial dilution method. Single spores located under microscope were transferred to water agar plates and incubated for further studies. Pathogenicity test was carried out by inoculating locally adapted susceptible french bean cultivars Him1 and Contender.

Method of inoculation

Seeds of test cultivars, Him1 and Contender were surface sterilized with 90% ethyl alcohol for 10 minutes and were sown in plastic pots of 20cm size. Inoculation was done at 3-4 trifoliate stage by spraying inoculum containing 1x10^6 conidia of M. phaseoli per ml on surface of the leaves. Inoculated plants were placed in the growth chamber for 3-4 days at 22±1°C with more than 90% RH. These plants were transferred to the net-house and observed for the appearance of disease symptoms.

Isolation and pathogenicity studies of Xanthomonas axonopodis pv. phaseoli

Infected samples of french bean leaves were collected from the field and surface sterilized with mercuric chloride (0.1%) for 25-30 seconds and then were cut into small bits. After washing in few changes of sterile distilled water, bits were cut with a sterilized blade on a clean slide containing a few drops of water and allowed to stand for a minute to enable bacterial cells to ooze out. Three Petri plates were streaked with a loop full of bacterial suspension. These plates were incubated at 27+ 1°C in the B.O.D. incubator for 24h. Single colonies of 24 h old bacterial growth were transferred to the nutrient agar (NA) slants under aseptic conditions. The bacterium produced yellow colonies with brown pigmentation. The pathogen was maintained on NA and yeast extract chalk agar media by sub-culturing at 6-7 day intervals.

Method of inoculation

Healthy seeds of the test cultivars Contender and Him1 were sterilized with 90% ethyl alcohol and sown in plastic pots. Fifteen-day-old plants at 3-4 trifoliate leaf stages were inoculated with bacterial suspension in water with inoculum density of 5x 10^8cfu/ ml by using carborundum cotton-swab method. Inoculated plants were kept in glass-house where day temperature varied between 24-34°C with more than 90% RH. The plants were regularly observed for appearance of symptom expression.

Evaluation of Phaseolus spp. for disease resistance

Various accessions / lines of P. vulgaris, P. acutifolius and P. coccineus were evaluated (Table 1) for resistance to floury leaf spot and bacterial blight under field conditions at three locations during two consecutive years namely Palampur (1200m), Patlikuhl (1500m), and Sangla (2500m).

Interspecific hybridization

Inter-specific crosses were attempted at Palampur (Kangra), Patlikuhl (Kullu), and Sangla (Kinnaur), latter two locations ranging from 1500m to 2500 m above mean sea level representing three different agro-climatic zones of Himachal Pradesh. Contender, Him1 (P. vulgaris), Pc1, Pc2 and Pc5 (P. coccineus) and G 40001, G 40013, G 40015, G 40017 and G 40020 (P. acutifolius) were used in the present studies where P. vulgaris was used as female parent. Emasculation and pollination was done as per method described by Honma (1956).

<table>
<thead>
<tr>
<th>Common name</th>
<th>Line/ Accession number</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scarlet runner bean</td>
<td>Pc1, Pc2, Pc3, Pc4 and Pc5</td>
<td>Sangla (Kinnaur, H.P.)</td>
</tr>
<tr>
<td>(Phaseolus coccineus)</td>
<td>G35005, G35059, G35413, G 35443</td>
<td>CIAT, Cali, Colombia</td>
</tr>
<tr>
<td>Tepary bean</td>
<td>G40001,G40013,G40015, G 40020</td>
<td>CIAT, Cali, Colombia</td>
</tr>
<tr>
<td>(Phaseolus acutifolius)</td>
<td>G 40017,G 40020</td>
<td>H.P. Krishi Vishva-vidyalaya, Palampur (H.P.)</td>
</tr>
<tr>
<td>Common bean</td>
<td>Contender, Him-I</td>
<td></td>
</tr>
<tr>
<td>(Phaseolus vulgaris)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Evaluation of interspecific hybrids for resistance

Interspecific hybrids obtained from the crosses between *P. vulgaris* and *P. coccineus* and *P. acutifolius* were evaluated for resistance to floury leaf spot and bacterial blight under natural epiphytotic conditions at Patlikuhl because both the diseases appear here in epidemic form every year. However, for bacterial blight artificial inoculation was also done on the plants grown under field conditions. Data were recorded on disease parameters like lesion size, lesion number and disease severity (0-5 scale). Area under disease progress curve (AUDPC) and infection rate was also calculated.

Scoring of disease reaction

a) Floury leaf spot

Data on floury leaf spot were recorded as disease severity using following 0-5 scale. 0; No disease, 1; 1-20% leaf area infected, 2; 21-40% leaf area infected, 3; 41-60 % leaf area infected, 4; 61-80% leaf area infected, 5; more than 80 % leaf area infected (James, 1971).

b) Bacterial blight

Data on terminal disease severity of bacterial blight were recorded on the basis of the following 0-5 scale. 0; No disease, 1; 1 -10% of leaves with blight lesions, 2; 11-25% of leaves with blight lesions, 3; 26-50% leaves with blight lesions, 4; 51-75% leaves with blight lesions, 5; more than 75% leaves with blight lesions (James, 1971). Plants with 0 and 1 score were categorized as resistant.

Progress of disease in time

Disease progress in time was studied by recording the severity of floury leaf spot and bacterial blight from appearance of first disease symptoms at weekly intervals. The rate of disease progress was calculated by computing the values in the following equation (Van der Plank, 1968).

\[
\text{Infection rate}(t) = \frac{2.3}{t_2 - t_1} \log_{10} \frac{X_2}{1-X_2} - \log_{10} \frac{X_1}{1-X_1}
\]

where

- \(X_1\) = disease severity at time \(t_1\)
- \(X_2\) = disease severity at time \(t_2\)

Area under disease progress curve (AUDPC) of interspecific hybrids was calculated by the following formula:

\[
\text{AUDPC} = \sum \frac{b_i + Y_{i+1} - Y_i}{2} \times dt_i
\]

where \(dt_i\) is the time interval between every two observations of \(Y_i\) and \(Y_{i+1}\).

Statistical analysis

Analysis of data was done using standard statistical methods (Gomez and Gomez, 1984). Randomized block design was used in experiments. The significance of difference was tested at 5% level of probability.

RESULTS

Isolation, pathogenicity and maintenance of pathogens

*M. phaseoli*

The pathogen was cultured on water agar. Small young colonies of 1-2 mm across sporulated slightly. Inoculation of susceptible cultivars Contender and Him1 at different crop growth stages produced no typical symptoms of the disease. Therefore all evaluation work against this pathogen was done under natural epiphytotic conditions.

*X. axonopodis pv. phaseoli*

In yeast extract chalk agar, bacterial mass multiplication was more in comparison to that of nutrient agar. The pathogen produced brown pigment in both media within 4 days of incubation at 27+1°C in BOD incubator. It proved pathogenic on the locally grown cultivars Him1 and Contender. It was found to be rod shaped and gram negative.

Evaluation of alien germplasm for resistance to pathogens

*P. acutifolius* and *P. coccineus* lines were free from floury leaf spot. All tepary bean lines were resistant to both the pathogens at Palampur and Patlikuhl, however, there was no germination of seed in Sangla (Table 2). All the lines of *P. coccineus* except Pc3 and Pc 4 were resistant to
fuscos blight. In Contender disease severity was between 21.3 to 28.4% and 18.4 to 30.6% for floury leaf spot and bacterial blight while it ranged between 16.9 to 24.5% and 20.3 to 32.1% for Him1 for both the diseases respectively when tested at three different locations.

**Interspecific hybridization**

Most of the crosses set pods but majority of them failed to reach maturity and dropped within 3-5 days of pollination. In some cases pods reached 2-3 cm in length, turned yellow and finally abscised. However, a few crosses namely Contender x Pc2 (3), Contender x G 40015 (1), Contender x G 40020 (1), Him1 x Pc1 and Him1 x Pc2 (7) resulted in pod setting which reached maturity and ultimately seed formation. The number of successful crosses was highest at Palampur (9) followed by Palampur (4) and Sangla (1).

**Evaluation of hybrids for resistance to pathogens**

1. **Floury leaf spot**

   Almost all the inter-specific hybrids were found resistant to the pathogen. Floury leaf spot in some hybrids appeared 10-15 days late as compared to check cultivars. Lesion number varied from 0-12 with maximum lesion size of 2.60 mm$^2$ on the upper surface and 3.60 mm$^2$ on lower surface (Table 3). In susceptible cultivar Contender, lesion number varied from 11-15 with lesion size of 2.80 mm$^2$ on the upper surface and 4.60 mm$^2$ on the lower surface whereas on Him1 lesion size varied between 3.60 mm$^2$ on upper surface and 4.80 mm$^2$ on the lower surface. Terminal disease severity in the interspecific hybrids ranged from 0 to 2.1% whereas on susceptible checks Contender and Him1 it was 11.4 and 20% respectively. Area under disease...
progress curve (AUDPC) for the susceptible checks Contender and Him1 was 93.5 and 201, which was quite low for the hybrids and ranged from 0 to 21.5. Similarly infection rate was also low in the hybrids (0-0.05) against 0.11 for the susceptible checks.

**II. Bacterial blight**

Thirteen inter-specific hybrids were evaluated for resistance to fuscous blight. Not much variation could be observed for lesion number among susceptible checks and interspecific hybrids. Larger lesions were observed on the hybrids *P. vulgaris* x *P. acutifolius*, however, lesion size on the hybrids of *P. vulgaris* x *P. coccineus* was comparatively very small (56-77 mm²) than Contender (260 mm²) and Him 1 (336 mm²). Maximum lesion size of 240 mm² was recorded in hybrid from cross *P. vulgaris* Contender x *P. acutifolius* G 40015. In later stages of growth, there was an increase in the number of lesions and sharp decrease in lesion size. Number of blight lesions was less in hybrids in comparison to check cultivars. Terminal disease severity was recorded as mean of all leaves.

<table>
<thead>
<tr>
<th>Interspecific cross/ parental line</th>
<th>Lesion No.</th>
<th>Average lesion size (mm²) Upper leaf surface</th>
<th>Lower leaf surface</th>
<th>Disease severity* (%)</th>
<th>AUDPC</th>
<th>Infection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contender x G 40015</td>
<td>0*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Contender x G 40020</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Him1 x Pc1</td>
<td>6.0</td>
<td>1.60</td>
<td>2.34</td>
<td>1.6</td>
<td>16.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Him1 x Pc2</td>
<td>5.0</td>
<td>2.00</td>
<td>3.20</td>
<td>1.1</td>
<td>7.7</td>
<td>0.06</td>
</tr>
<tr>
<td>Him1 x Pc2</td>
<td>7.0</td>
<td>2.10</td>
<td>3.20</td>
<td>1.8</td>
<td>15.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Him1 x Pc2</td>
<td>12.0</td>
<td>2.60</td>
<td>3.60</td>
<td>2.1</td>
<td>21.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Him1 x Pc2</td>
<td>5.0</td>
<td>1.20</td>
<td>2.00</td>
<td>1.5</td>
<td>10.5</td>
<td>0.05</td>
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<tr>
<td>Contender</td>
<td>11.0</td>
<td>2.80</td>
<td>4.60</td>
<td>11.4</td>
<td>93.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Him1</td>
<td>15.0</td>
<td>3.60</td>
<td>4.80</td>
<td>20.0</td>
<td>201.0</td>
<td>0.11</td>
</tr>
<tr>
<td>Pc1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pc2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G 40015</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G 40020</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Disease severity was recorded as mean of all leaves **0 means no disease

Area under disease progress curve (AUDPC) for hybrids was comparatively low and ranged from 21.5 to 392.6 whereas susceptible lines Contender and Him1 exhibited larger AUDPC up to 988.6 and 636.9 respectively (Table 4). The infection rates in some hybrids were also low in comparison to susceptible parents. In the resistance donor parents, AUDPC and infection rates were comparatively much less. The infection rates in inter-specific hybrids obtained from *P. vulgaris* Him1 x *P. coccineus* Pc1 or Pc2 were very low whereas in other hybrids derived form crosses between *P. vulgaris* Contender and *P. acutifolius* G 40015 or G 40017 were similar to the susceptible parents.

**DISCUSSION**

This paper presents the first report on transfer of resistance in french bean to floury leaf spot from *P. coccineus* and *P. acutifolius*. There are a few reports of partial resistance to bacterial blight transferred from alien germplasm to french bean...
Some workers have correlated resistance to photoperiod and temperature conditions (Coyne et al., 1973). Schuster et al. (1983) advocated that several factors should be taken into consideration in evaluating resistance to X. axonopodis pv. phaseoli. Variation in the virulence of X. axonopodis pv. phaseoli has been observed frequently and isolates from tropical regions appear to be more virulent than those from temperate areas (Schuster et al., 1973). In the present study resistant donor parents (P. coccineus and P. acutifolius) were screened for resistance against floury leaf spot and fuscous blight along with locally grown frenchbean cultivars Contender and Him1 at three different locations. Resistance was observed in the donor species at all the three locations, hence selected for hybridization to transfer resistance in the locally grown commercial cultivars.

Hybrids formed in the present studies were evaluated for resistance to both the pathogens at Patlikuhl as the inoculum load was more than Palampur and Sangla. Floury leaf spot in hybrids appeared 10-15 days late as compared to check cultivars. P. vulgaris Contender x P. acutifolius G 40015 and P. vulgaris Contender x P. acutifolius G 40017 were disease free while remaining hybrids involving the cross (P. vulgaris x P. coccineus) exhibited varying number of lesions, but the disease level was quite low than the checks. There was increase in halo size with increase in lesion size of floury leaf spot. These results are in conformity with the findings of Sharma et al. (1996). Level of resistance to bacterial blight in the hybrids of P. vulgaris x P. acutifolius is comparatively very low than the hybrids of P. vulgaris x P. coccineus which shows resistance to bacterial blight can be successfully transferred from P. coccineus to locally adapted cultivars of P. vulgaris. Allington and Chamberlain (1949), Skoog (1952) have reported that tolerant beans possess factors that keep the bacterial populations down. Resistance has successfully been transferred from tepary bean (Honma, 1956; McElroy, 1985; Scott and Michaels, 1988) and runner bean (Freytag et al., 1982; Park and Dhanvantary, 1987) to common bean. Although high level of resistance to floury leaf spot has been transferred from P. acutifolius in the present study, however, P. coccineus has been found good source for transfer of resistance against both the pathogens.

Our results therefore confirm the strong possibility of transferring resistance from alien sources to P. vulgaris against a variety of pathogens. Interspecific hybrids possessing high level of resistance obtained in the present study can be effectively used in the further breeding programme.

<table>
<thead>
<tr>
<th>Interspecific cross/ parental line</th>
<th>Lesion No.</th>
<th>Average lesion size (mm²)</th>
<th>Disease severity* (%)</th>
<th>AUDPC</th>
<th>Infection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contender x G 40015</td>
<td>12</td>
<td>240</td>
<td>34.3</td>
<td>392.6</td>
<td>0.07</td>
</tr>
<tr>
<td>Contender x G 40020</td>
<td>6</td>
<td>187</td>
<td>21.5</td>
<td>221.9</td>
<td>0.10</td>
</tr>
<tr>
<td>Him1 x Pc1</td>
<td>24</td>
<td>70</td>
<td>8.2</td>
<td>184.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Him1 x Pc2</td>
<td>21</td>
<td>77</td>
<td>6.7</td>
<td>109.0</td>
<td>0.06</td>
</tr>
<tr>
<td>Him1 x Pc2</td>
<td>20</td>
<td>88</td>
<td>6.4</td>
<td>119.1</td>
<td>0.05</td>
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<tr>
<td>Him1 x Pc2</td>
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<td>56</td>
<td>8.6</td>
<td>121.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Him1 x Pc2</td>
<td>14</td>
<td>70</td>
<td>7.2</td>
<td>174.6</td>
<td>0.04</td>
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<td>Parental Line</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contender</td>
<td>19</td>
<td>260</td>
<td>44.0</td>
<td>636.9</td>
<td>0.10</td>
</tr>
<tr>
<td>Him1</td>
<td>26</td>
<td>336</td>
<td>55.3</td>
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<td>70</td>
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<td>0.04</td>
</tr>
<tr>
<td>G 40020</td>
<td>8</td>
<td>126</td>
<td>10.5</td>
<td>155.7</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Disease severity was recorded as mean of all leaves

Table 4. Field evaluation of interspecific hybrids (F₁s) of french bean for resistance to fuscous blight

(Thomas and Waines, 1984; Zapata et al., 1985).
aiming to develop frenchbean varieties resistant to fuscous blight and floury leaf spot which are a serious problem in the bean growing areas of the state.

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