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Characterization of *Pseudomonas savastoni* pv. *phaseolicola* in Seed lots of Mung bean (*Vigna radiata* L. Wilczek) collected from Rajasthan, India

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Abstract

Halo blight caused by Pseudomonas savastoni pv. phaseolicola (Pph) of mungbean is an important seed-borne bacterial disease accounting for a heavy loss in its production worldwide. In the present study, a total of 151 seeds were collected from fields of 14 districts of Rajasthan state and screened for occurrence and percent incidence of Pph. The pathogen was isolated from infected and healthy looking seeds. A total of 30 isolates were found and identified by growth on a semi selective agar medium MSP, LOPAT tests and biochemical characteristics such as sugar utilization, gelatin liquefaction, H_2S production and esculin hydrolysis etc. medium MSP was used for isolation and confirmation of the pathogen. The higher incidence of Pph on MSP was recorded in seed samples of Ajmer (50- 93%), Bhilwara (42- 71%), Churu (35-69%), Ganganagar (48-59%), Jalore(54-79%), Jhunjhunu(37-71%) and Nagaur(43-88%) districts.

Keywords: Biochemical characteristics, Halo blight, Incidence, LOPAT tests.

Introduction

Mung bean is a rich source of vitamins, minerals and proteins due to which it is popular in developing countries[1-3]. AVDRC in its report declared it as valuable crop as it fix the nitrogen from the air into nitrate which is a helpful component for plant growth and soil fertility[4] which has contributed in expansion of opportunities for employment, diversification of diets and also helped in increasing nutritional security in Asia[2]. It suffers from a large number of bacterial diseases.

Pseudomonas savastoni pv. *phaseolicola* (Pph) causes halo blight which is severe in areas with cold and humid climate of developed and developing countries like India[5]. It is a seed borne bacterial disease[6,7] and its pathogenic host range varies among pulses from *Vigna radiata* to *Vigna mungo*, *Vigna ungulata*, *Phaseolus vulgaris* and *P. Lunata*[8]. The disease is characterized by development of water–soaked lesions surrounded by a halo at 18-20°C temperature[9-11]. The pathogen remains in seeds and activates on their germination[10,12]. The severity and threat of the disease may be realised by the fact that it is considered to be a big threat in US and a law has been passed to destroy the entire crop if found with Pph infection[13].

Material and Methods

Field survey and collection of samples: A total of 151 seed samples were collected from fields of 14 districts of Rajasthan state which are producing mung bean at higher scale. Infected leaves, stems and pods were also collected from fields and potted plants.

Isolation, purification, identification and characterization: The collected seed samples were categorised as bold discoloured, moderately discoloured and healthy looking on the basis of appearance of discolouration of cotyledon, on and around hilum and wrinkles on seed coat by dry seed inspection. After surface sterilization with 2% sodium hypochloride (NaOCl) seeds with severe discolouration and shrivelling were directly plated on nutrient agar (NA) medium and incubated for 2-3 days at 25±2°C. Colonies showing typical morphological features of Pph were purified on NA by serial dilution and spreading. Purified colonies were subjected to LOPAT [Levan formation, Oxidase test, Potato soft rot test, Arginine hydrolysis[14] and Tobacco hypersensitivity test[15]] test and some other biochemical tests such as KOH solubility test[16], Gram's staining by Hucker's modification method, catalase test, gelatine liquefaction[14], H_2S production, starch hydrolysis and esculin hydrolysis for their characterization[17]. The pathogen was also streaked on KB (King's medium B) for production of fluorescence under UV light[18].

Isolates were also tested in Hellmers broth for utilization of sorbitol, erythritol, m-inositol and mannitol[19], and in Ayer's solid medium for utilization of L- tartarate, L-lactate and D-tartrate[20].

Similarly leaves, stems and pods having symptoms of Pph were surface sterilized, cut into pieces and transferred on NA. Suspected colonies were subjected to various biochemical and physiological tests for purification and characterization.

For identification of the pathogen, suspected colonies and seed samples were transferred on semi-selective medium modified

sucrose peptone agar medium (MSP). Colonies of Pph developed on MSP medium were raised circular, glistening, globose and slight yellow with less dense centre[18,21,22].

Pathogenicity and host test: Further the pathogen was tested for pathogenicity by development of typical symptoms of halo blight i.e. water soaked necrotic lesions on detached leaves of tobacco and host plant. Suspension of 24 hrs old culture of the pathogen was inoculated in the mid rib of leaves with the help of a sterilized hypodermal needle. Inoculated leaves were incubated for 2-3 days at 25±2°C for symptoms development[23]. Leaves of *Vigna radiate, V. mungo*, *V. Ungulate* and *P. vulgaris* plants were used for host test.

Results and Discussion

Halo blight of mung bean has been identified as a big threat to the crop worldwide. A total of 151 seed samples were collected from 14 districts of Rajasthan, categorised in bold discoloured, moderately discoloured and healthy looking seeds and examined for occurrence and incidence of Pph in the present study but in some seeds no visible symptoms were seen at all but as also reported earlier in reports of Buruchara et al.[24] but when sown they produced symptomatic seedlings.

Total 30 isolates from seeds were purified from seeds of mung bean in this study. All the isolates were gram negative and rod shaped, positive for KOH solubility and fluorescent on KB medium. It has been reported that Pph is levan +ve, oxidase -ve, not able to utilize sugars such as mannitol, sorbitol and inositol as sole carbon source whereas some strains were able to utilize mannitol[11,25]. In the present study also the pathogen was positive for levan production on SNA medium, tobacco hypersensitivity test and showed negative response for oxidase production, potato softening and rotting. Similarly it was found to be non-responsive for arginine in medium, H_2S production and esculin hydrolysis whereas positive for starch hydrolysis and catalase activity as testified earlier[26,27]. The pathogenicity test revealed that all isolates were able to cause halo blight symptoms on *Vigna radiata* and other host leaves. All the isolates of Pph produced creamy or white circular colonies on NA and raised circular, glistening, globose and slight yellow with less dense centre on MSP as mentioned in guideline of ISTA[18].

The seeds from seed samples of different districts were placed on semi-selective medium MSP for incidence of Pph. 115 seed samples revealed the pathogen. The higher incidence of Pph on MSP was recorded in Ajmer (50- 93%), Bhilwara (42- 71%), Churu (35-69%), Ganganagar (48-59%), Jalore (54-79%), Jhunjhunu (37-71%) and Nagaur (43-88%) district in 42, 4, 5, 4, 4, 3 and 11 seed sample respectively. However, relatively a moderate incidence was recorded in Tonk (17-52%), Sikar (30-59%), Pali (30-74%), Jodhpur (21-65%), Jaipur (22-68%), Hanumangarh (27-53%) and Barmer (23-57%) districts in 6, 4, 9, 5, 7, 6 and 5 seed samples respectively.

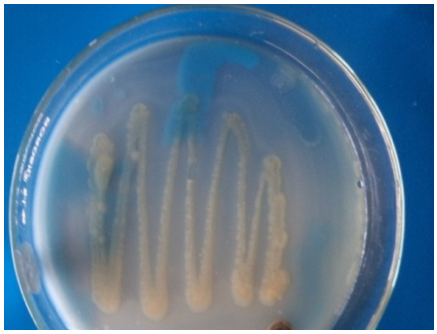
Conclusion

In present study 151 mung bean seed samples from 14 districts of Rajasthan state were screened for the presence of Pph. The pathogen was isolated on nutrient agar and streaked on semi-selective medium MSP for further confirmation. The pathogen was rod shaped and Gram's negative. The presumptive pathogen was characterized biochemically and pathogen was found positive for gelatin liquefaction, levan formation, catalase test and starch hydrolysis while negative for oxidase test, arginine hydrolysis, casein hydrolysis, esculin hydrolysis, H_2S production and not able to utilize sugars such as mannitol, sorbitol and inositol as sole carbon source but some isolates were able to utilize mannitol.

Table- 1
Biochemical characteristics of different isolates of *P. syringae* pv. *phaseolicola*

Characteristics	Isolates (30)	Reference (Pph)
Gram's reaction	-	-
KOH solubility test	+	+
Gelatin liquefaction test	+	+
Kovac's oxidase test	-	-
Catalase test	+	+
Arginine dihydrolysis	-	-
H_2S production	-	-
Esculin hydrolysis	-	-
Starch hydrolysis	+/-	+
Casein hydrolysis	-	-
Growth temperature	23-27◦C	23-27◦C
Hypersensitivity test	+	+
Utilisation of [1] -		
D-Mannitol	-/+	-
D-Sorbitol	-	-
L-Lactate	-	-
D+Tartrate	-	-
L+Tartrate	-	-
Erythretol	-	-
Inositol	-	-
Host test[2]	+	+

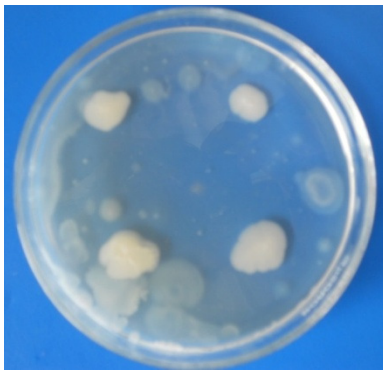
A: Development of fluorescence on KB medium under UV light by pathogen;



B: Colonies of Pph with no zone of hydrolysis and creamy white and flat appearance on MT medium.

C: Colonies of Pph are light yellow with less dense centre, glistening raised globose on MSP mdeium;

D: Complete hydrolysis of starch by Pph



E: Domed and globose colonies (Levan formation) of Pph on SNA medium;

F: Gelatin liquefaction test of pathogen, note liquefaction of gelatin in test tube inoculated with pathogen in comparison to control (left);

G: Brown necrotic symptoms caused by Pph on leaf of tobacco plant

Figure- 1 (A-G)
Physiological methods for diagnosis and identification of *P. savastoni* pv. *phaseolicola*

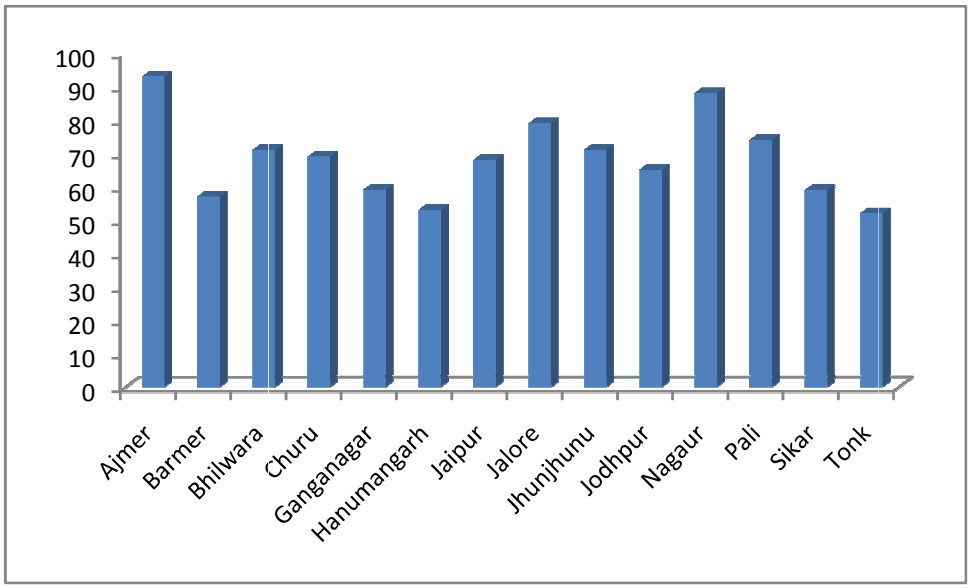


Figure-2
Incidence (Maximum value) of Pph in seed samples collected from 14 districts of Rajasthan: Ajmer district has shown highest (93%) and Tonk district has shown least incidence (52%) in the present study

The pathogen was able to produce necrotic symptoms on tobacco leaves and leaves of host plants in pathogenecity test. Although the pathogen was recorded in more than 76% seed samples studied, the districts of Ajmer (50- 93%), Bhilwara (42-71%), Churu (35-69%), Ganganagar (48-59%), Jalore (54-79%), Jhunjhunu (37-71%) and Nagaur (43-88%) revealed its relatively higher incidence.

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