

Full Length Research Paper

Survey, surveillance and cultural characteristics of bacterial blight of soybean

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A survey was undertaken in eight districts (Parbhani, Nanded, Hingoli, Beed, Osmanabad, Jalna, Latur and Aurangabad) of Marathwada region during June to August in Kharif, 2009 to 2010. In all, 69 soybean fields were surveyed (roving survey) for recording the severity and incidence of soybean blight. The most serious pod disease was noticed on the soybean field of Parbhani district, followed by Hingoli, Nanded, Latur and Beed. The variety JS-335 showed the maximum pod blight severity in all surveyed districts. The average disease incidence was 14.5% in Marathwada region. The highest disease incidence (23%) was noticed in Parbhani district, followed by Hingoli (20%), Nanded (17%), Latur (15%) and Beed (13%). The lowest disease incidence (7%) was noticed in Jalna district. The pathogen was isolated and purified. Its pathogenicity was proven in pot culture. Further, on the basis of morphological, cultural characteristics of the pathogen and symptomatology, the fungal (*Pseudomonas syringae* is bacteria) pathogen was identified as *Pseudomonas syringae*.

Key words: Survey, bacterial blight, soybean, *Pseudomonas syringae*.

INTRODUCTION

Soybean belongs to the family Leguminosae and sub-family Papilionoidae. The crop is widely grown in the world and is a primary source of vegetable oil and protein. It contains 44.65% protein, 8.77% fats, 27.12% nitrogen, 5.89% ash, 5.96% fibre. In India, the area, production and productivity of soybean during 2007 to 2008 are 79.720 lakh ha., 64.28 lakh metric tonnes and 802 kg/ha, respectively (Anonymous, 2007).

Soybean crop is known to be affected by more than 100 plant pathogens. Among them, 35 plant pathogens are of economic importance. Bacterial blight caused by *P. syringae* has been reported to be the major disease in the production of soybean. It causes yield losses ranging from 4% to as high as 40% under extreme condition (Meshram and Sheo-raj, 1988, 1992; Lim, 1992; Mishra and Krishna, 2001). Considering the variable types of wilt reactions of the released variety in the farmer's field, sick

plot at different locations and yield losses, the investigation was undertaken to find out the major causal organism involved in chickpea wilt complex in Marathwada region of the Maharashtra state. Survey and surveillance of chickpea wilt complex incidence on farmer's field was done. Furthermore, the wilt pathogens were isolated and purified, and its pathogenicity was confirmed.

MATERIALS AND METHODS

Survey and surveillance

A roving disease survey of the incidence and severity of bacterial blight disease of soybean during July to August in Kharif 2009 was undertaken at Parbhani, Nanded, Hingoli, Beed, Osmanabad, Jalna, Latur and Aurangabad districts of Marathwada region. Total sixty nine soybean fields were inspected out of which number 11, 5, 7, 11, 6, 9, 10 and 10 were from Parbhani, Jalna, Aurangabad, Hingoli, Osmanabad, Beed, Nanded and Latur districts, respectively. Latur and Nilanga in Latur district, Aurangabad and Vijapur in Aurangabad District, Parbhani, Selu, Purna and Pathri in

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Parbhani district, Nanded and Bhokar in Nanded district, Hingoli and Basmat in Hingoli district, Beed, Kaij and Ambejogai in Beed district, Osmanabad and Tuljapur in Osmanabad district and Jalna and Badnapur Tahsils in Jalna district were inspected for recording the incidence and severity of bacterial blight disease. Each spot 5 to 6 plants were surveyed. Incidence of bacterial blight disease was examined as severe, moderate, trace and free, on the basis of percentage of severity of disease. Similarly, the percent disease incidence of bacterial blight was calculated by using formula.

$$\text{Percent disease incidence} = \frac{\text{Number of plant infected}}{\text{Total number of plant examined}} \times 100$$

$$\text{Percent disease intensity (PDI)} = \frac{\text{Sum of all disease rating}}{\text{Number of ratings} \times \text{Maximum disease grade}} \times 100$$

Isolation of blight pathogen

Soybean plants (JS-335) naturally infected with typical blight symptoms were collected from farmers' field and brought to the laboratory. All samples collected from different locations were subjected to isolation of blight pathogen on nutrient agar (NA) in the laboratory.

Pathogenicity test of blight pathogen

Five seeds of the susceptible variety JS-335 were sown in each pot filled with soil and FYM in 2:1 proportion and immediately watered with sterilized distilled water. After germination, only four seedlings were maintained in each pot. When the plants were 4 weeks old, the bacterial suspension (10^8 cfu/ml) was prepared as the inoculum for pathogenicity test. The underside of the leaf was sprayed with water and dusted with carborendum powder. Further, these leaves were smeared with bacterial suspension by means of sterile cotton swab. These pots were placed in a glasshouse, where high humidity and optimum temperature ($24 \pm 2^\circ\text{C}$) were maintained for further development of bacterial blight disease symptom. After eight days incubation period, typical symptoms on foliage of artificially diseased (bacterial blight) soybean plants were observed. The microbe was reisolated on nutrient agar medium.

Cultural studies of pathogenic bacteria

Growth characters of the isolated *P. syringae* was studied by growing it on different nutrient agar culture media, including nutrient agar, potato dextrose agar, yeast extract mannitol agar and glucose chalk agar medium. These agar media were prepared by following standard laboratory procedure, sterilized by autoclaving, poured into the sterile petri plates, (ten plates of each medium) and allowed to cool down and solidify. Then, the plates were inoculated by bacterium and incubated at room temperature.

RESULTS

Survey of bacterial blight severity of soybean in different districts of Marathwada region

The results revealed that the severity of disease was

maximum in Parbhani district, followed by Hingoli, Nanded, Latur and Beed. The minimum disease severity was observed in Jalna district (Table 1 and Figure 1). The result also showed that the variety JS-335 was highly susceptible to disease in all districts, followed by MAUS-81 and MAUS-71 (Table 1).

Incidence of bacterial blight of soybean in different districts of Marathwada region

The results (Table 2 and Figure 2) revealed that the maximum percent disease incidence (23%) was observed in Parbhani district, followed by Hingoli, (20%), Nanded (17%), Latur (15%), Beed (13%), Aurangabad (11%) and Osmanabad (10%). The minimum percent disease incidence (7%) of bacterial blight of soybean was found in Jalna district. This result was similar to Song et al. (2002). The soybean yield was reduced substantially due to this disease. The average percent disease incidence in Marathwada region was about 14.5%. In all, sixty nine fields were surveyed and average disease incidence (PI) to the tune of 14.5% has been observed.

Pathogenicity

In the initial stage, the small, irregular lesions (yellow to brown spots) were produced. The lesions dried out, turned reddish brown to black and became surrounded by a yellowish green halo. Results showed that young leaves were more susceptible to the bacterial infection.

Identification of bacterial pathogen

The pathogen isolated from the diseased plants was identified on the basis of the morphological characters as *P. syringae* and was confirmed from NIKU, BIO-Research Lab and PUNE-411002. The results in respect of the growth characters of *P. syringae* on various culture media are presented in Tables 3 to 5. The results revealed that the moderate growth was obtained on medium yeast extract mannitol agar broth and glucose chalk agar broth of susceptible variety JS-335. All the three media were proved to be significantly superior in favoring the growth of *P. syringae* to the rest of the media tested. The poor growth was observed on nutrient broth. Abundant growth, filmform nature, glistening, butyrous, pale yellow fluorescent colonies were observed on nutrient agar medium *in vitro*. Fairly good growth, filiform, slightly raised, glistening, whitish yellow fluorescent, unbanate, secondary colonies were observed on PDA medium. Also, filiform, glistening, yellowish fluorescent filiform, fairly good growth were observed on chalk agar medium *in vitro*.

Table 1. Survey of bacterial blight severity of soybean in different districts of Marathwada region.

S/N	District	Number of field surveyed	Variety	Disease severity				
				Severe	Moderate	Trace	Free	Total
1	Parbhani	11	JS-335	2	2	1	-	5
			MAUS-81	1	1	1	-	3
			MAUS-71	-	1	1	1	3
			Total	3	4	2	1	11
2	Nanded	10	JS-335	1	2	-	1	4
			MAUS-81	1	1	1	-	3
			MAUS-71	1	1	1	-	3
			Total	3	4	2	1	10
3	Hingoli	11	JS-335	2	2	1	1	6
			MAUS-81	1	1	1	-	3
			MAUS-71	1	1	-	-	2
			Total	4	4	2	1	11
4	Beed	9	JS-335	1	1	2	-	4
			MAUS-81	-	1	1	1	3
			MAUS-71	1	1	-	-	2
			Total	2	3	3	1	9
5	Osmanabad	6	JS-335	1	-	1	1	3
			MAUS-81	1	1	1	-	2
			MAUS-71	-	1	-	-	1
			Total	2	2	2	-	6
6	Jalna	5	JS-335	1	1	1	-	2
			MAUS-81	-	1	1	-	2
			MAUS-71	-	1	-	-	1
			Total	1	3	2	-	5
7	Latur	10	JS-335	2	1	1	1	5
			MAUS-81	1	2	-	-	3
			MAUS-71	-	1	1	-	2
			Total	3	3	1	-	10
8	Aurangabad	7	JS-335	1	1	1	-	3
			MAUS-81	1	2	-	-	3
			MAUS-71	-	1	-	-	1
			Total	2	3	1	-	7

Severe : > 50%; Moderate : > 10% to < 50%; Trace : < 10%.

DISCUSSION

The symptoms typically resembled those previously reported by Nishiyama et al. (1986) and Verma (1995) (1994). Reisolation studies revealed the presence of the same fungus (bacteria) identical to the original one obtained from natural bacterial blight plants. The

morphological and cultural characteristics of the *P. syringae* obtained after reisolation were similar to those reported earlier by several workers Akhtar and Khan (1988), Li young Hao et al. (1995) and Supriadi et al. (1996). Two weeks after germination, leaves of seedling in each pot were smeared with bacterial suspension by means of sterile cotton incubation period, typical small yellow spots

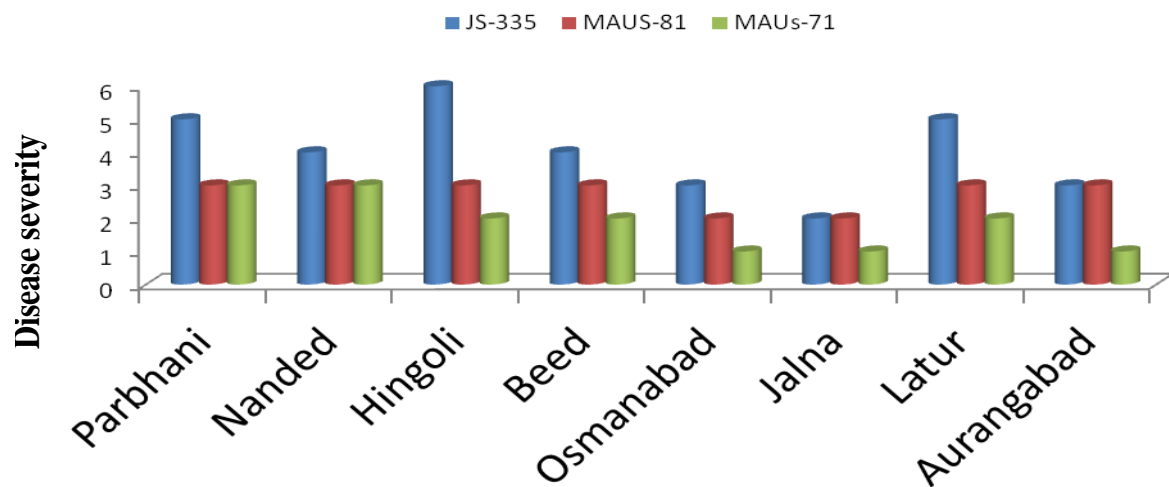


Figure 1. Survey of bacterial blight severity of soybean in different districts of Marathwada region.

Table 2. Percent disease incidence of bacterial blight of soybean in different districts of Marathwada region.

S/N	District	Total number of field surveyed	Total number of plants examined	Infected plants with bacterial blight	Disease incidence (%)
1	Parbhani	11	300	67	23
2	Nanded	5	200	14	17
3	Hingoli	7	250	27	20
4	Beed	11	300	30	13
5	Osmanabad	6	200	20	10
6	Jalna	9	250	32	7
7	Latur	10	250	47	15
8	Aurangabad	10	250	37	11
Average					14.5

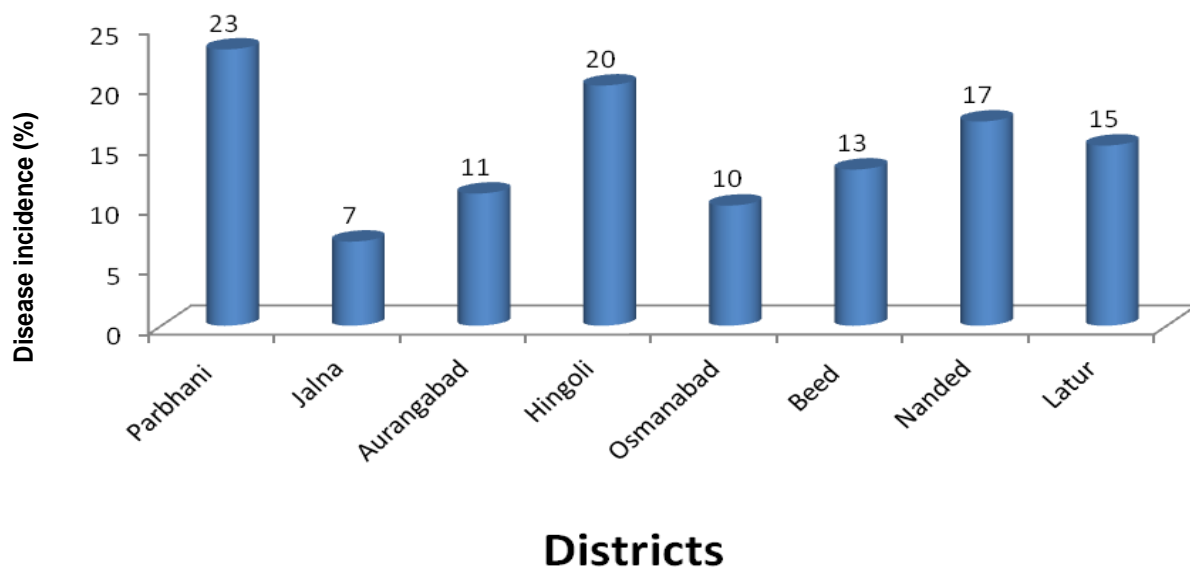


Figure 2. Percent Disease incidence of bacterial blight of soybean in different districts of Marathwada region.

Table 3. Growth and cultural characters of *Pseudomonas syringae* pv. *glycinea* on different agar media in petri plate.

S/N	Medium	Growth characters after 48 h of incubation
1	Nutrient agar	Growth abundant, filiform, slightly raised, glistening, butyrous, pale yellow fluorescent secondary colonies begin to develop along the margin
2	Potato dextrose agar	Growth fairly good, filiform, slightly raised, glistening, whitish yellow fluorescent, unbanate, secondary colonies begin to develop along the margin
3	Yeast extract mannitol agar	Growth abundant, colonies were filiform, citron yellow, fluorescent raised circular entire margin, secondary colonies begin to develop along the margin
4	Glucose chalk agar medium	Growth fairly good, filiform slightly raised with entire margin, glistening, pale yellow, fluorescent slightly turn brown

Table 4. Growth and cultural characters of *Pseudomonas syringae* pv. *glycinea* on different agar slants.

S/N	Medium	Growth characters after 48 h of incubation
1	Nutrient agar	Colonies of first were filiform and light yellow fluorescent then becoming waxy yellow, growth was abundant
2	Potato dextrose agar	Colonies at first were light yellow, fluorescent filiform, unbanate good growth.
3	Yeast extract mannitol agar	Colonies were filiform, citron yellow, fluorescent growth was abundant.
4	Glucose chalk Agar Medium	Colonies were filiform, slight brown colour, growth was abundant

Table 5. Growth and cultural characters of the *Pseudomonas syringae* pv. *glycinea* in different broth.

S/N	Name of broth	Surface growth	Turbidity	Amount of growth
1	Nutrient broth	Slight growth, slightly fluorescent	Light cloudy	Poor
2	Potato dextrose broth	Membranous	Light cloudy	Scanty
3	Yeast extract mannitol agar broth	Pellicle	Light cloudy	Moderate
4	Glucose chalk Agar Broth	Pellicle	Light cloudy	Moderate

on leaves were observed. Highest disease incidence and severity were noticed in Parbhani district followed by Hingoli, Nanded, Latur and Beed while lowest disease incidences were noticed in Jalna district. The cultural studies results obtained are in agreement with the findings of Song et al. (2002).

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