Prevalence of Green gram leaf blight caused by *Macrophomina phaseolina* (Tassi) Goid

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***Abstract*— During the survey, occurrence of leaf blight disease in greengram was noticed in serious proportion inflecting heavy losses around Navsari, Gujarat, India. Cultivar GM-2K-5 was found more severely affected in the area during kharif and summer seasons. The repeated isolations from infected leaves revealed the association of Macrophomina sp., which was identified as Macrophomina phaseolina (Tassi) Goid. The pathogenicity was proved by different artificial inoculation methods with positive results.**

***Index Terms*— Greengram, Macrophomina phaseolina, isolation, pathogen**

I. INTRODUCTION

Greengram (Phaseolus aureus Roxb.) is one of the important pulse crops, primarily grown for food in India. Disease is the major constraint in economic crop production as they inflict heavy losses. Like other crops, greengram is also attacked by many diseases during seed germination to seed production and maturity. Over 35 fungal pathogens, few viral, bacterial pathogens and nematode species are known to attack greengram resulting into substantial yield losses (Agrawal, 1989). During the survey, occurrence of leaf blight disease in greengram was noticed in serious proportion inflecting heavy losses around Navsari, Gujarat, India. Cultivar GM-2K-5 was found more severely affected in the area during kharif and summer seasons. The repeated isolations from infected leaves revealed the association of Macrophomina sp., which was identified as Macrophomina phaseolina (Tassi) Goid. The pathogenicity was proved by different artificial inoculation methods with positive results. The information on the disease is meagerly available and this disease looks becoming as a limiting factor in the cultivation of greengram in south Gujarat and hence creating information on leaf blight of greengram will be very useful.

Considering the seriousness of the problem, the present investigation was carried out to pinpoint exact cause and to find out suitable control measures for the disease.

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II. MATERIAL AND METHODS

A. Collection of samples

The diseased leaves of greengram showing typical, well developed dark brown spots and blightening along the margin were collected from the Pulse Research Station, Navsari Agricultural University, Navsari. The infected leaves were brought into the laboratory, placed in blotting papers under pressure with herbarium press and preserved for further investigations. The symptoms and signs observed in nature were critically recorded.

B. Isolation of the pathogen

Fresh infected leaves of green gram showing typical, well developed dark brown spots and blightening along the margin were used to isolate the pathogen from the infected area. The infected area was cut into small pieces in such a way that each piece comprised of infected as well as healthy tissues. The pieces were surface sterilized with 0.1 per cent mercuric chloride (HgCl2) solution for 30 seconds followed by three washing with distilled water and then transferred aseptically using laminar air flow system (cabinet manufactured by Klenzoid contamination control Ltd.) on sterile petriplates containing 20 ml potato dextrose agar (PDA) medium (peeled potato 200g, dextrose 20g and agar agar 20g in 1000ml distilled water) and these petriplates were incubated at room temperature. The fungal hyphae developing from the infected tissue were sub-cultured aseptically on PDA slant and pure culture thus obtained was maintained. This culture was microscopically examined for identification and further purified by using single hyphal tip isolation technique and the culture obtained was maintained on PDA slant for further investigations.

C. Identification of the pathogen

Identification of pathogen causing leaf blight of green gram was carried out by studying the cultural and morphological characters were recorded right from initiation of growth till the period of 15 days. The morphological characters viz., mycelial growth and sclerotial formation were studied under low power magnification from 10 days old culture of Macrophomina phaseolina and were compared with those given in literature. The microphotograph of sclerotia was also taken. The pure culture was also sent to Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi-110 012 for confirmation and identification.

D. Pathogenicity Test

To know the pathogenic nature of Macrophomina phaseolina isolated from blightened greengram leaf sample, it was tested on one-month-old healthy greengram plants raised in sterilized earthen pots. The mycelial suspension was

prepared from the 8 days old culture of Macrophomina phaseolina by homogenization of culture in distilled sterile water. The pots were watered up to saturation in the morning and the inoculation was carried out in the evening. The leaves were surface sterilized with 0.1 percent mercuric chloride solution and washed thoroughly with distilled sterile water to remove traces of the mercuric chloride. The inoculation was carried out on leaves by four methods.

1) Pin prick method
2) Tooth brush injury
3) Injury by carborandum powder
4) Without injury

In above (1-4) methods, the pathogen was inoculated on leaves by rubbing sterile cotton swab dipped in mycelial suspension of M. phaseolina and in fourth method the suspension was sprayed on the leaves by hand atomizer without making injury. Suitable controls with only distilled water spray were maintained. All the plants were then kept under moist chamber, which were wetted thrice a day to provide sufficient humidity around the plants. Observations with regard to infection and symptoms development were recorded. The fungus was reisolated from the inoculated diseased leaves and the morphological and cultural characters were compared with those of M. phaseolina, which was previously isolated from diseased greengram leaves.

III. RESULTS AND DISCUSSION

Disease is a major constraint in economic crop production as they inflict heavy losses. In greengram, diseases are considered as a complex nature caused by various pathogens. Among the various diseases affecting greengram, leaf blight caused by Macrophomina phaseolina (Tassi) Goid. has became a severe threat to successful and profitable cultivation of greengram in south Gujarat. The variety, GM-2K-5 was found susceptible to the leaf blight causing severe yield loss.

Pathological investigation
Symptomatology

The symptoms of leaf blight of greengram were recorded from suscetible Cv. GM-2K-5 grown at Pulse Research Station, N.A.U., Navsari. The symptoms and signs produced in natural conditions were recorded which are as under.

In the initial stage of infection, small, circular to irregular, dark brown to reddish brown or black lesions appeared on or near the margins of the leaves, which enlarged and coalesced, spots gradually spreading into a large irregular necrotic area with brown periphery and gray coloured centre. Eventually 'Shot Hole' symptoms appeared. The leaf blight started from the margin of the leaf and proceeds inward. In severe infection, necrosis of lesions ensured covering major leaf area which finally resulted in drying up and defoliation of the affected leaves. In severe conditions, the infection was also recorded on pod and stem. Dark brown spots were observed on pod. Later, dark brownish fungal growth covering the pods and seeds were also noticed. Seeds become shriveled and blackened. Leaf blight appeared on 4 to 6 weeks old plants. Usually, the older leaves were first attacked. Severely affected leaves fall off prematurely. The disease was observed during kharif as well as summer but was more progressive and severe during the months of September to October in kharif and April to May in summer.

The systemic studies on leaf blight (M. phaseolina) symptoms of greengram under field condition revealed that it was by and large similar to the description provided by the earlier workers where Philip (1968), Grover and Sakhuja (1981) and Agrawal (1989) described the symptomatology of leaf blight of mung bean caused by M. phaseolina.

Collection of samples and isolation of pathogen

The diseased leaves of greengram showing typical well developed dark brown spots with gray coloured center and blightened margins with dark brown colour were collected from the Pulse Research Station, N.A.U., Navsari. The symptoms and signs were observed visually and the presence of the pathogen was confirmed by microscopic examination.

The diseased leaves collected from infected field were subjected to repeated tissue isolation on PDA medium after confirming the presence of pathogen by microscopic examination. Isolation from diseased leaves yielded Macrophomina sp., which was maintained on PDA slants and used throughout the investigation.

A. Identification of the pathogen

After purification of the fungus as described under materials and methods, morphological and cultural characters of the fungus grown on PDA were studied for identification and compared those mentioned in literature. The pure culture was also sent to Indian Type Culture Collection (I.T.C.C.), Division of Plant Pathology, I.A.R.I., New Delhi-110 012 and was identified as Macrophomina phaseolina (Tassi) Goid. The fungus also produced leaf blight symptoms under pathogenicity test. The studies on the morphological and cultural characters of isolated Macrophomina sp. showed its close identity with Macrophomina phaseolina (Tassi) Goid. as described by Agrawal (1989) and Nakarni (1991). There was no pycnidial formation in any of the media tested. This result supports the results obtained by Vidhyasekharan and Arjuna (1978) and Grover and Sakhuja (1979). Thus, the causal organism of greengram leaf blight under present investigation confirmed as Macrophomina phaseolina (Tassi) Goid.

B. Morphological characters

The colonies of M. phaseolina grew fast in PDA and attained diameter of 8.7 cm within 5 days at room temperature with profused mycelial growth and sclerotial formation. The fungus produced initially white mycelial growth on PDA later changing to brown black in centre due to the formation of numerous small black sclerotia. The mycelium was hyaline to brown, branched, septate and 1.78 to 6.58 µm in width. The sclerotia formed in culture were black, hard and 67.92 to 195.18 µm in diameter. The pycnidial stage was not produced in culture. The morphology of the pathogen studied here is in line with the morphology of M. phaseolina causing leaf blight of greengram as described by Agrawal (1989).

C. Pathogenicity test

The pathogenicity of M. phaseolina was carried out by four methods viz., pin prick, injury by tooth brush, injury by carborandum powder and without injury. It is evident from the results and symptoms produced that the pathogenicity was proved. The leaf blight pathogen, M. phaseolina was able to infect and develop the symptoms on greengram leaves

irrespective of inoculation methods. Spraying with sterile water (control) produced no disease symptoms.

Typical symptoms became evident after seven days of inoculation as brown to black spots on the leaf. Later on, these spots enlarge, coalesce, resulting into blightening of leaves. Re-isolation from artificially inoculated diseased leaves yielded Macrophomina sp., which was identified with original one.

Typical symptoms of leaf blight of mung bean (M. phaseolina) developed, which were similar to those described by Philip (1968) and Grover and Sakhuja (1981) during pathogenicity test. Satyaprasad et al. (1981) have also proved pathogenicity of M. phaseolina causing leaf blight of Pepromia thomsoni by these methods. Present findings are also on the same line and confirmed the pathogenicity of the organism isolated.

IV. CONCLUSION

The typical leaf blight symptoms observed in the field were small, circular to irregular, dark brown to reddish brown or black lesions, appeared on or near the margins of the leaves, which enlarged and coalesced, spots gradually spreading into a large irregular necrotic area with brown periphery and gray coloured center and resulted in "Shot Hole" symptoms. The leaf blight started from the margin of the leaf and proceed inward. In severe infection, necrosis of lesions ensured covering major leaf area which finally resulted in drying up and defoliation of the affected leaves. Microscopic examination and tissue isolation from leaf of infected plant yielded culture of Macrophomina sp. The morphological and cultural characters of Macrophomina sp. isolated were studied, which were found closely identical with Macrophomina phaseolina (Tassi) Goid. and this was also confirmed through identification by Indian Type Culture Collection, Division of Plant Pathology, I.A.R.I., New Delhi. The pathogenicity test carried out by pin prick, injury by both tooth brush and carborendum powder and without injury on leaves. All these methods successfully produced typical leaf blight symptoms similar to those observed under natural condition and described in literature and confirming pathogenic nature of the fungus. Thus, the causal agent of greengram leaf blight identified and confirmed as Macrophomina phaseolina (Tassi) Goid.

REFERENCES

[1]Agrawal, S.C. (1989). "Diseases of green gram and black gram". International Book distributors, pp. 1-2,5,159,181,269,28-29,32-33.

[2]Grover, R.K. and Sakhuja, P.K. (1981). Some pathological studies on Rhizoctonia bataticola leaf blight of mungbean. Indian Phytopath., 34: 24-29.

[3]Nakarni, N.R. (1991). Investigation on M. phaseolina (Tassi) Goid., causing root-rot of castor. M.Sc. (Agri.) Thesis, Gujarat Agril. University, Sardar Krishinagar.

[4]Philip, C.T. (1968). Studies of Rhizoctonia disease of mung at Jabalpur. M.Sc. (Agri.) Thesis. JNKVV, Jabalpur, M.P., India, 70 pp.

[5]Satyaprasad, K.; Usharani, P. and Ramarao, P. (1981). A new leaf spot of pepromia caused by M. phaseolina. Indian Phytopath., 34(2): 237.

[6]Vidhyasekharan, P. and Arjunan, G. (1978). Studies on leaf blight of urdbean caused by M. phaseoli. Indian Phytopath., 31(3): 361-362.