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SYMPTOM EXPRESSION, ISOLATION, PURIFICATION, PATHOGENICITY AND MORPHOLOGICAL IDENTIFICATION OF *RHIZOCTONIA BATATICOLA*

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ABSTRACT

The charcoal rot of soybean is one of important disease it cause 70% loss in soybean yield. The disease is common in M.P., Maharashtra, Rajasthan, Uttaranchal, Punjab and Delhi. The ten isolates of *Macrophomina phaseolina* were collected during month of June to September, 2022 from the soybean fields of Agriculture Research Station, Ummedganj (Kota). The sand sorghum medium (SSM) was used for mass multiplication (mass inoculum) of *Rhizoctonia bataticola* in laboratory. The pathogenicity test of isolates of *Macrophomina phaseolina* isolated from the different parts of the Agriculture Research Station, Ummedganj (Kota) was tested by using susceptible cultivar JS-20-34.

Keywords : Charcoal rot, pathogenicity test, *Macrophomina phaseolina*, soybean.

Introduction

Soybean (*Glycine max* L.), a legume crop native to Eastern Asia, is cultivated extensively in the United States of America, Brazil, China, Russia, and India. United States is the single largest producer of the soybean in the world. Brazil and Argentina stand second and third biggest producers of soybean respectively, Among Asian countries, China and India stands at fourth and fifth position respectively for soybean production in the world. (Anonymous, 2016).

In India, major soybean-producing states include Madhya Pradesh, Maharashtra, Rajasthan, and Uttar Pradesh. Recognized as the “Golden Bean,” soybean is valued for its diverse applications in food, feed, and industry, and is considered a promising crop for addressing global protein deficiency. Primarily grown during the *kharif* season, its growth period coincides with the monsoon months, making it vulnerable to a range of foliar, soil-borne, seed-borne, and seed-transmissible diseases.

Soybean suffers from several biotic stresses but root rot caused by *Rhizoctonia bataticola* (Taub.) Butler is among the five top most important diseases causing huge annual losses to crop (Wrather *et al.*, 2001). Notable diseases affecting soybean include charcoal rot (*Macrophomina phaseolina*), purple blotch (*Cercospora kikuchii*), pod rot and collar rot (*Fusarium semitectum*), seedling and pod blight (*Colletotrichum truncatum*), soybean rust, leaf spot (*Alternaria alternata*), and bacterial pustule, among others. Root rot caused by *Rhizoctonia bataticola* (Taub.) Butler (Pycnidial stage *Macrophomina phaseolina*) is one of the economically important disease of soybean (Yang and Navi, 2005).

In India, root rot disease (*R. bataticola*), which was previously supposed to be minor disease of soybean and other crops. But during recent past, it has become a serious disease, which may be due to altered weather conditions, particularly longer and repeated droughts during crop growth period (Lodha and Mawar, 2010; Gupta *et al.* 2012b).

Materials and Methods

Collection of diseased samples

Infected plant samples which showing typical collar rot symptoms were collected during month of June to September, 2022 from the soybean fields of Agriculture Research Station, Ummedganj (Kota). Samples were brought in Department of Plant Pathology, College of Agriculture, Ummedganj, (Kota) for isolation and further studies.

Isolation of fungus

The part of root showing typical symptoms of disease was cut into small pieces. Then these pieces were surface sterilized with 0.1% HgCl₂ solution for one minute. Such pieces were washed thoroughly in sterile distilled water three times to remove the traces of mercuric chloride solution, and then aseptically transferred to sterilized potato dextrose agar (PDA) plates. They were incubated at 25±1°C for three days for growth of fungus. Later, the bit of fungal growth was transferred to PDA slants. The pure culture of fungus was obtained by further growing culture and following hyphal tip culture under aseptic conditions. As method suggested by (Aneja, K. R., 2018). The pure cultures were maintained on PDA slants at 4±1°C for further studies.

Identification of pathogen

The pathogen *Rhizoctonia bataticola* forms cottony white colonies on PDA. The colonies appeared as dull white to pure white mycelial growth and turn into dark coloured mycelial initiation of 8-9 days of incubation. On the basis of these characters pathogen was identified as *Rhizoctonia bataticola*. Further, pathogen was identified from ITCC (Indian Type Culture Collection) Lab, IARI, Division of Plant Pathology, New Delhi.

Maintenance of the culture

The fungus *Rhizoctonia bataticola* was sub-cultured on PDA slants and allowed to grow at 25±1°C temperature in the incubator for ten days. The culture was obtained stored in a refrigerator at 4±1°C and sub cultured once in a month.

Pathogenicity test

Sterilized soil was filled in cemented pots of size 45 x 30 cm². Fifteen days old culture grown on sorghum grains 20g mass culture of *Rhizoctonia bataticola* grown on sorghum seeds was added to the soil in pots and mixed thoroughly with soil to get sick soil. Then healthy seeds of root rot susceptible soybean variety (JS20-34) were soaked overnight in water and surface sterilized with 0.1% HgCl₂ solution, washed

thrice in tap water before sown in the pots containing sterile soil. Pots without *Rhizoctonia bataticola* culture served as control. Moisture content in pot was maintained to field capacity by adding required amount of water periodically. Observations on germination, pre and post emergence mortality were recorded on. Re-isolation was made from such affected portion of the plant tissue and compared with that of original culture.

Results and Discussion

Symptoms

The infected soybean plants showed overall yellowing followed by drooping of leaves at initial stage and later on dried off. Plants bearing leaves appeared weak and dark lesions on the bark at ground level was also noticed in field plots appeared first as reddish-brown lesions on hypocotyls close to cotyledons, then turned ashy-gray to black. Mycelial inoculation was almost equally effective in causing seedling mortality. Affected seedlings were pulled up easily and underground parts softened and rotted which left in the soil (Plate 1).

Isolation

Isolation of the pathogen was carried out successfully from infected diseased roots of soybean by applying tissue isolation technique on PDA. Observations on morphology of fungus were recorded from seven days old culture grown on PDA. The fungus initially produced white circular shaped colonies with septate mycelium which later turned dark grey to black in colour. Characteristic right angled branching of hyphae and constriction at the point of branching was also noticed. The fungus was purified by single hyphal tip method and were maintained separately on PDA slants stored at 4°C in a refrigerator and mass multiplication of the test pathogen was done by using Sand-Maize medium for further *in vitro* studies. The current findings were in accordance with the earlier reports of Sharma *et al.* (2015), Lakhran *et al.* (2018), Gade *et al.*, (2018) isolated *R. bataticola*, from infected soybean samples by tissue isolation technique on PDA medium and found fluffy, linear mycelium. Gaikwad *et al.*, (2020) collected dry root rot diseased chickpea plants and carried out isolation of *R. bataticola*, by subjecting to tissue isolation on PDA, Premalatha *et al.* (2020) also isolated *R. bataticola* from diseased specimens of various host plants.

Identification

The pure culture obtained from tissue isolation was purified by using hyphal tip method and was maintained on PDA slants for further studies. The pure culture thus obtained was identified as *Rhizoctonia bataticola* on basis of morphological characters.

Further, the identification of pathogen was also confirmed as *Rhizoctonia bataticola* from Indian Type of Culture Collection, Division of Plant Pathology, IARI, New Delhi (Ref. No. 22-23/PP/663; Date-05/09/2022; ID-11,720.22).

Pathogenicity

Pathogenicity of *R. bataticola* following Koch's postulates was confirmed by applying sick soil method in pot culture under greenhouse conditions by sowing seeds of soybean cultivar JS-20-34. Leaves became yellow and the petioles kept the leaves attached to the stem. The symptoms showed up 30 days after inoculation and were similar to those seen in naturally infected soybean plants. The affected plants dried out after four to five days and tissue shredding was a noticeable symptom in these dried plants.

The pathogenicity test revealed constant association of *R. bataticola*, with root rot disease of soybean. Re-isolation of the fungus from artificially root rot diseased specimens of soybean consistently yielded *R. bataticola* on PDA plates and their morphological and cultural characteristics exactly matched with *R. bataticola* pure cultures isolated from naturally root rot diseased specimens of soybean. Baste *et al.* (2016) conducted pathogenicity test of 9 isolates of *R. bataticola* causing root rot of soybean collected from Vidarbha region. The per cent mortality was observed up to 83.33% for different isolates. Artificially inoculated plants expressed typical symptoms of root rot. The infected plants loss turgidity and showed chlorotic symptoms. Gade *et al.* (2018) tested pathogenicity of *R. bataticola* causing dry root rot of soybean by sick soil method and noted to 80% seedling mortality. He was also proved the pathogenicity of *Rhizoctonia solani* caused root rot disease in soybean. Deshmukh *et al.* (2022) tested the seeds of susceptible soybean cultivar TAMS-38 were used for studying the pathogenicity of isolate in sick pot culture. In the pathogenicity test, *R. bataticola* found pathogenic to soybean causing 80% root rot disease. The soybean TAMS-38 exhibited root rot on 30-35 days after sowing. Jejurkar *et al.* (2022) proved pathogenicity of the test pathogen *R. bataticola* caused root rot of soybean Cv. JS 335 by sick soil method in pot culture under screen house conditions.



Plate 1 : (a) Root of Healthy plants and (b) Root of infected plants



Plate 2 : Mycelium of *Rhizoctonia bataticola*



Plate 3 : Pure culture of *R. bataticola* (a) initial white growth of pathogen, (b) later turn into carbonaceous brown to black.



Plate 4 : Mass multiplication *R. bataticola*



Plate 5 : Pathogenicity test (i) Un-inoculated (ii) Inoculated with *R. bataticola*.

Conclusion

Root rot disease in soybean was characterized by initial yellowing of foliage followed by leaf drooping. Infected seedlings could be easily uprooted, and their underground tissues were found to be softened and rotted, remaining in the soil. The causal pathogen was successfully isolated from diseased soybean roots on potato dextrose agar (PDA) medium and identified as *Rhizoctonia bataticola* (Taub.) Butler by ITCC, IARI, New Delhi. The culture was purified using the single hyphal tip method, and pure isolates were maintained on PDA slants under refrigeration for further investigations. Pathogenicity was confirmed on soybean cultivar JS-20-34 through the sick soil technique under greenhouse conditions.

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