Research Article

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STANDARDIZATION OF METHODOLOGY FOR STUDYING PATHOGENECITY OF *XANTHOMONAS CAMPESTRIS* PV ORYZAE TO RICE

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Abstract: Rice is the most important food crop of India and its production has increased significantly during last few years. However, Infection of *Xanthomonas campestris* pv. Oryzae considerably decrease its productivity. It is important to study pathogenecity of causative organism before any defense strategy is decided. Present paper deals with standardization of technology for microscopic study of pathogen.

Keywords: *Xanthomonas campestris*, Rice, Disease

INTRODUCTION

Rice is the most important cereal food crop of India. It occupies about 23.3% of gross cropped area of the country. It plays vital role in the national food grain supply. Rice contributes 43% of total food grain production and 46% of the total cereal production of the country. Rice is the staple food of more than 60% of the world's population especially for most of the people of South-East Asia (13). Among the rice growing countries in the world, India has the largest area under rice crop and ranks second in production next to China(11).Rice production in India is nearly 91.05 million tons during 2001-02,95.69 during 2007-08 and 98.99 during 2008-09(13, 14). Plants are hosts to thousands of infectious diseases caused by a vast array of phytopathogenic fungi, bacteria, viruses, protozoa and Nematodes. It is clear that wide-spread development of a major rice disease would be disadvantageous to the world's economy. The direct agronomic consequence of such an event would be a severe decrease in crop yield and stability of production.

Bacterial blight caused by *Xanthomonas campestris* pv *oryzae* is the one of the serious disease of rice 1st noted in 1884 in Japan (11). This disease is occurred globally from Asia to Africa and America. Its distribution ranges from 200 S in Queensland Australia to 580 N in Heilang Jiange China, and from sea level to the Tibetan Plateau (1, 11) In India this disease was reported 1st time in 1951 on Khopoli area of Mumbai.Bacterial Blight disease is known to routinely occur in rice-growing states like Andra Pradesh, Bihar, Haryana, Kerala, Orrisa, Punjab and Uttar Pradesh. The disease occurs during the August, September and October and with a total rainfall of at least 20mm (5,6).

These bacteria - *Xanthomonas campestris* pv *oryzae* spread rapidly from rice plant to rice plant and from field to field in water droplets. Infected leaves develop lesions, yellow and wilt in a matter of days. Main symptoms of bacterial blight is kresk and leaf blight. This organism share three characteristics: (1) it colonize in the intercellular spaces of rice plants and is capable of killing plant cells; (2) It is host specific in host plants, and produce various symptoms after several days of multiplication and (3) in non-host plants and in resistant plant it trigger the hypersensitive response (HR), arapid, defense-associated, programmed death of plant cells at the site of invasion.

MATERIALS AND METHODS

T(N)1 and CRRI-XA21 varieties, which are susceptible and resistant to bacterial leaf blight were collected from Central Rice Research Institute, Cuttack, Orissa and Birla Agriculture University Ranchi, Jharkhand. These rice genotypes were further tested by pathogenesis test and observed for the susceptibility and resistant to the pathogen.

Collection of pathogenic bacteria:

Xanthomonas campestris pv *oryzae* Strain no 5028 was also collected from NCIM Pune grown on MGYP (Malt extract-0.3g, Glucose-1.0g, Yeast extract-0.3g, Peptone-0.5g, Agar-2g, pH-6.4-6.8, Distilled water-100ml) media at 370C in incubator for 3-6 days and pathogenesis test was done. After pathogenesis test was done. After pathogenesis test this is to notice that this strain is virulent for the rice plant so this strain is selected for the further study.

Standardization of Pathogen Infection:

Xanthomonas campestris pv *oryzae* a virulent strain was grown on MGYP (Malt extract-0.3g, Glucose-1.0g, Yeast extract -0.3g, Peptone-0.5g, Agar-2g, pH-6.4-6.8, Distilled water-100ml) media at 370 C in incubator for 3-

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6 days. After incubation bacterial cell was counted by haemocytometer and the selected suspension was inoculated by syringe filter method on the 14, 25 and 30 days old rice plants. The above said bacterial cell suspension was adjusted to 2x108 cells/ml and was inoculated. Different methods like (a) Clip cut method (b) Dip method (c) Syringe filter method were chosen for the inoculation. *X. oryzae* pv. Oruzae growth in planta was measured using a modified method from a report by Song et al. (Song W *et al.,*). For establishing growth curves, the inoculated rice leaves were harvested at each time point, immediately sliced into small pieces. Sliced rice leaves were incubated in 1 ml sterile water including 15ug/ml of cephalexin with shaking for 1h, and then filtered through two layers of cheesecloth. The filtrates were then plated onto MGYP plates with cephalexin for5028 strain. Colonies on the plates were counted after three days of incubation at 3.

Microscopic study between Host Pathogen:

To visualize bacterial infection through leaf veins under microscope, rice leaves were harvested at 14DAI.To get thin transverse section (0.1 mm), TN1 and CRRI-XA21 leaves inoculated with pathogen and cut using a razor blade and stained with Fluorescent dye Acridine Organe. The small pieces of leaf sections were placed on a microscope slide, submerged in immersion oil (Cargille lab, USA), covered with a glass slip and sealed with grease. The fluorescence photographs were taken using a Leica FW 4000 fluorescent microscope fitted with fluorescein isothiocyanate filters (excitation filter, 450 to 490 nm; emission filter, 520 nm; dichroic mirror, 510nm). The optimal exposure time was 1 sec. Three biological replicates using leaf blades from each of the three different plants were used for all microscopic analyses.

RESULTS

Standardization of growth conditions of plant material:

In this study different varieties of rice (blight resistant and susceptible) were grown in different pots under the desired condition. We noticed that there is some morphological changes are occurred on the leaves of rice which was inoculated with bacteria but there are no changes in the control plant.

Standardization of pathogen infection:

The pathogenicity of the *Xanthomonas campestris* pv *oryzae (5028)* was tested on the susceptible rice line IR24 and the near-isogenic lines. Rice plants were inoculated by the clipping method (Kauffman *et al.,* 1973). Three to four leaves were used per plant and one leaf per rice line was clipped using H2O for control. Five to six plants were inoculated per isolate and were kept for 18 h in humid chambers (>_ 92% relative humidity) at 30+ 40C, and were thereafter brought back to greenhouse conditions for disease development. Fourteen days after inoculation, symptoms were evaluated by measuring the lesion lengths of the leaf covered by bacterial leaf blight lesion. Plants were divided into three classes: resistant, with lesion length of 0-3cm; and susceptible, > 9cm. No lesions were observed in control experiments in which the leaves were inoculated with scissors dipped in water. We found the clip cut method is best for the infection. It was clear from the above result; lesion length was less in the resistant and more in the susceptible rice plant. From the above result it evident that the resistant variety have some genes present which cope the invading of bacterial pathogen and project themselves during pathogen attack. The function of these defense-related proteins is to inhibit the growth of the attacking pathogens and provide resistance to plants (15, 16).

Microscopic Study between Host and Pathogen:

We examined if the multiplication and colonization of bacteria can be visualized in rice leaf tissue. TN1 and CRRI-XA21 were inoculated with the 5028 strain, and then at 14 DAI a small segment of the leaf five centimeters down from the inoculation site was harvested. The leaf tissue was cut into segments with a rozor blade, and the transverse sections of the leaf were observed under a fluorescent microscope. The 5028 strain propagated along vascular systems and was clearly observed. These results demonstrate that the 5028 strain proliferated in the susceptible lines but not in resistant lines and that the difference can easily be detected using fluorescent microscopy. Our observation of 5028 in the xylem is consistent with the findings of previous studies showing that *X. oryzae* pv. *Oryzae* is a vascular pathogen (Nino-Liu DO *et al.,* Mew TW).

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