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Serological detection of *Xanthomonas oryzae pv. Oryzicola,* The Bacterial Leaf Streak Pathogen In Leaves And Seeds Of Rice Plant

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ABSTRACT

Bacterial leaf streak of rice caused by *Xanthomonas oryzae pv. oryzicola* is a seed borne pathogen. Development of antiserum against the pathogen will help to detect the pathogen in seed lot. In the present study, the polyclonal antiserum raised against purified *X. oryzae pv. oryzicola in* New Zealand white rabbit was used to detect the pathogen present in leaves and seeds of rice plants. The titre value of polyclonal antiserum raised against *X. oryzae* pv. *oryzicola* was measured by indirect ELISA. Both the antigen and antiserum could be diluted upto 1:100; when they were diluted beyond 1:100, they were not reactive to each other. The antiserum raised against *X. oryzae* pv. O*ryzicola* could detect the bacterium from the seed and leaves, but it did not react with8 *X.* oryzae pv. oryzae infected leaves thus proving that the antiserum produced for *X. oryzae pv. oryzicola* is very specific to bacterial leaf streak pathogen alone.

Key words : Bacterial leaf streak, Oryzae sativa, serological detection, *Xanthomonas oryzae pv. oryzicola.*

INTRODUCTION

Bacterial leaf streak of rice caused by *Xanthomonas oryzae pv. oryzicola* is one of the most serious diseases of rice. It is a widely distributed disease in India, particularly in Andhra Pradesh, Bihar, Eastern Madhya Pradesh and Orissa Rao *et al.,* (1993). Seed borne nature of this pathogen significantly intensifies the spread of this disease Zhou *et al.,* (2003).

Introduction of enzyme- linked immunosorbent assay (ELISA) has been an important land mark in serological detection and assay of viral and bacterial plant pathogens. It has become a preferred method because of its sensitivity, economical use of antiserum, availability of quantifiable data, and the capacity for rapid handling of large number samples. Polyclonal and monoclonal antibodies have been successfully used to detected the bacterial leaf streak pathogen in rice Zhang *et al.,* (1996); Xu *et al.,* (1990) and Benedict *et al.,* (1989). Development of antiserum against the *X. oryzae pv. oryzicola* will help to detect the pathogen in seed lot and this will avoid tedious and time consuming grow out tests. In the present study, polyclonal antiserum raised against purified *X. oryzae pv. oryzicola* in New Zealand white rabbit was used to detect *X. oryzae* pv. *Oryzicola,* present in leaves and seeds.

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MATERIALS AND METHODS

Pathogen :

X. oryzae pv. *oryzicola* was isolated from bacterial leaf streak infected rice plant (Co 47) at paddy breeding station, Tamil Nadu Agricultural University, Coimbatore, India and pure culture of the pathogen was maintained on agar slants of Wakimoto semi synthetic medium at -10^{0}C. The culture was stored in sterile 30 percent (v/v) glycerol at 70^{0}C for long term preservation. Moore *et al.,* (1992).

Antigen preparation :

Log phase culture (36 h old) of *X. oryzae* pv. *oryzicola* was centrifuged at 13,000 g for 30 min. (4^{0}C). The resulted pellet was washed two times with 0.01M phosphate buffered saline (PBS) by repeated centrifugation at 8000 g for 10 min. (4^{0}C). The antiserum was developed for the total protein after sonication. So the resuspended pellet was sonicated with a Fisher Sonic Dismembrator Model 300 for 2 min. De Boer and Schead, (1993). The protein content was determined Bradford, (1976) and 200 ug of protein was used for each injection.

Antibody production :

Injection and blood collection : Antiserum was raised against *X. oryzae* pv. *oryzicola* in a New Zealand white rabbit through four intramuscular injections. Two hundred ug of protein in PBS (pH 7.4) was emulsified with an equal volume of complete and incomplete (alternatively) adjuvant. Totally four injections were given, at weekly interval followed