SUMMARY...
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Seeds play a very important role as a source of inoculum of pathogens causing serious diseases in important crop plants. Seeds are also carriers of pathogens and are known to cause epidemics of many diseases. Most of the bacterial diseases are seed borne and are of wide occurrence in tropical and sub-tropical regions including India and in particular Karnataka. Seed borne bacterial diseases like bacterial blight of paddy caused by Xanthomonas oryzae pv. oryzae, black rot of cabbage caused by Xanthomonas campestris pv. campestris, bacterial spot of tomato caused by Xanthomonas vesicatoria and leaf blight of cowpea caused by Xanthomonas axonopodis pv. vignicola have been selected in the present study.

The detection/identification of disease causing pathogens is a necessity for diagnosis and management of these diseases. Assays and techniques involving isolation on non-selective/semi-selective media, biochemical and physiological tests performed for the identification of the pathogen are time consuming requiring 4-5 weeks to complete.

In order to overcome these shortcomings, serological techniques such as ELISA, immunofluorescent technique are used for diagnosis of plant diseases.

In order to simplify the existing techniques and to reduce the test time period without compromising on sensitivity, efforts have been made in these investigations to suitably apply and modify these techniques.

Infected leaves and seeds of paddy, cabbage, tomato and cowpea were collected from the fields showing typical symptoms of the above described bacterial diseases. Totally 12 cultivars of paddy, 6 cultivars of
cabbage, 12 cultivars of tomato and 11 cultivars of cowpea were collected.

The pathogenic bacteria were isolated by Standard microbiological procedures and identified phytopathogen on the basis of Gram reaction, morphological, biochemical and pathogenecity tests. They were identified to be belonging to the genus Xanthomonas. Further Hypersensitive test on tobacco confirmed their pathogenic nature and pathogenecity tests on respective host plants confirmed Koch postulates. They were identified to be Xanthomonas oryzae pv. oryzae, Xanthomonas campestris pv. campestris, Xanthomonas vesicatoria and Xanthomonas axonopodis pv. vignicola respectively.

Antisera were raised against four bacterial species in rabbits, using the methods described by Vruggink and Geasteranus Mass (1975). The titre of the antisera were established by conducting tube agglutination tests and was found to be 1:60 for all the four phytopathogenic bacteria.

**Indirect Immunofluorescence Technique (IIF)**

All the four plant pathogenic bacteria Xanthomonas oryzae pv. oryzae, Xanthomonas campestris pv. campestris, Xanthomonas vesicatoria and Xanthomonas axonopodis pv. vignicola were detected in the naturally infected seeds of paddy, cabbage, tomato and cowpea respectively by Indirect Immunofluorescence Technique.

The pathogen viz., Xanthomonas oryzae pv. oryzae was detected in all the seed samples consisting of 100 and 200 seeds, each belonging to all the 12 cultivars of paddy. Cultivars Latha and Madhu recorded higher number of fluorescence cells i.e., 155.2 and 155.0 respectively for seed samples of 200 seeds, cultivar IR-64 was found to contain least number of cells of Xanthomonas oryzae pv. oryzae i.e., only 9 and 23.12 for seed samples of 100 and 200 seeds.
The efficiency of IIF was tested by comparing the number of fluorescent cells with dilution plating. Cultivars Latha and Madhu recorded a higher cell count of $6.70 \times 10^5$ and $6.50 \times 10^5$ respectively, for seed extracts of seed samples comprising 400 seeds indicating better correlation between the two techniques.

Similarly cultivar Express of cabbage recorded 88 and 145 fluorescence cells for seed samples comprising of 100 and 200 seeds indicating that lower number of cells detected in seeds reflect lesser number of cells revealed by dilution plating ($3.46 \times 10^4$ and $7.50 \times 10^5$ respectively).

Results were similar in seed samples of cultivars of tomato and cowpea. The results have been found to correlate well with cell count by dilution plating.

This is the first record of application of IIF in the detection of *Xanthomonas vesicatoria* in naturally infected tomato seeds. IIF is a very sensitive procedure for detecting and identifying plant pathogenic bacteria. Cell morphology can be visualized in IIF slides and it does not disturb the viability of the cells. Cells remain viable and they can be regrown from fluoroslides.

IIF has the advantage over IF in that the secondary antibody can be conjugated to the fluorescent dye (FITC), which can be used against any primary antibody.

**Immunofluorescence Colony Staining Technique**

Another very sensitive technique, which combines the advantage of dilution plating and Serology, is Indirect Immunofluorescence Colony Staining Technique. In this, the secondary antibody is tagged with the dye (FITC) and the minute colonies growing from cells trapped by the tagged antibodies fluoresce under UV light, which can be easily identified. This technique was applied to all the 12 cultivars of paddy, 6 cultivars of cabbage, 12 cultivars of tomato and 6 cultivars of cowpea.
with seed samples consisting of 100 and 200 seeds. Numerous fluorescent colonies showing typical apple green fluorescence numerous could be seen for all the 4 pathogens developed in the plates could not be counted. The cell population (cfu/ml) recorded were also high indicating high sensitivity of the technique. Similar findings are reported by earlier workers like Franken and van Vuurde (1989) who reported the sensitivity could be increased to 10 to 1000 folds. Some of the advantages or modifications achieved in the present studies are the use of crude polyclonal antisera, usage of small 35mm polystyrene petri plates instead of fluoroshies and use of hand held UV lamp instead of sophisticated equipment like fluorescent microscope. The time required for completion of test was also reduced by reducing the incubation time of the seed extract.

This is the first record of application of this technique with several modifications for the detection of seed borne phytopathogenic bacteria.

**Enzyme Linked Immunosorbant Assay (ELISA)**

The ELISA technique applied to the different cultivars of the four crop plants in which the bacteria were detected in the naturally infected seeds revealed that ELISA can be successfully applied for the detection of phytopathogenic bacteria carried in the seeds. Crude polyclonal antisera at dilution of 1:80 and HRPO conjugate at 1:1000 dilution and substrate OPD were used to carry out indirect ELISA for the detection of *Xanthomonas oryzae* pv. *oryzae*, *Xanthomonas campestris* pv. *campestris*, *Xanthomonas vesicatoria* and *Xanthomonas axonopodis* pv. *vignicola* in naturally infected seeds of paddy, cabbage, tomato and cowpea. A better correlation was noticed between extinction values and cfu/ml obtained by dilution plating. In case of paddy, cultivar Jaya (S6), extinction values of 0.115 correlated well with 148 x 10^4 cfu/ml obtained by dilution plating.
The results of IF and ELISA compared well with dilution plating. Seeds extracts recording higher cell count in dilution plating also showed a higher extinction value and IF count, these results are in agreement with reports of earlier workers (van Vuurde, 1987; Franken and van Vuurde, 1990; Norman and Alvarez, 1994; Khan, 1996 and Ravikumar and Khan, 1997) who reported that ELISA is less sensitive than IIF in the detection of seed borne plant pathogenic bacteria by the sensitivity of the technique with lower inoculum load.

**Immunoisolation**

In the present investigation, immunoisolation of the four seed borne phytopathogenic bacteria *viz.*, Xanthomonas oryzae pv. oryzae, Xanthomonas campestris pv. campestris, Xanthomonas vesicatoria and Xanthomonas axonopodis pv. vignicola, in the naturally infected seeds of respective host plants was carried out by selecting one cultivar from each crop. The technique could successfully detect the presence of phytopathogenic bacteria, in the seed extracts. AS dilution 1:400 was found to be more efficient in trapping the cells. Out of the two incubation time period, incubation overnight was more efficient than 1 hour incubation, since large no of colonies appeared in the test areas of the plates. This is the first record of application for detection of phytopathogenic bacteria in seeds.

**Immunosorption Dilution Plating (ISDP)**

This technique involves selective adsorption of bacterial cells in combination with dilution plating. All the four phytopathogenic bacteria could be detected and isolated by ISDP. Out of the four adsorbant used *viz.*, Gum Arabica, Protein-A, Farmvar and Nail polish, two AS dilution *viz.*, 1:100 and 1:200 and in combination with semi selective media used, ISDP has been found to be most efficient with a combination of
Nail polish, 1:200 AS dilution and semi selective media. Other workers (Stead et al., 1987, Ruissen et al., 1987) have also reported a 5-6 fold increase in percentage of colonies obtained.

This is the first time that this technique has been applied to detecting seed borne bacteria causing important diseases of crop plants and can be adopted in small Research stations/quarantine stations.

**Dot Immunobinding Assay (DIBA)**

Dot Immunobinding Assay can be compared to ELISA but it differs, that in DIBA the antigen is adsorbed to a solid matrix like a NCM or plastic sheets. In the present studies all the cultivars of the four crop plants have recorded positive reaction. It has been compared with ELISA and has been found to show better correlation. Cheap alternate adsorbing materials like filter paper Whatman No. 1 and 41 were tested and found to be very fragile in the conduction of DIBA and the colour spots became diffused during the steps of procedure.