Neth. J. Pl. Path. 98 (1992) 169-178

Comparison of immunofluorescence microscopy and dilution-plating for the detection of Xanthomonas campestris pv. campestris in crucifer seeds

A.A.J.M. FRANKEN

Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, the Netherlands

Accepted 23 March 1992

Abstract

The correlation between immunofluorescence microscopy (IF) and dilution-plating on nutrient starch cycloheximide agar (NSCA) or NSCA with the addition of nitrofurantoin and vancomycin (NSCAA) was studied for the detection of *Xanthomonas campestris* pv. *campestris* (Xcc) in crucifer seeds. When checking 50 µl of the seed extract in IF, IF and dilution-plating gave corresponding results (both positive or negative) for 45.4-56.4% of the samples tested. No differences were observed in this respect between tests using a polyclonal antiserum (PCA 94) and replicate tests using monoclonal antibodies (MCA 20H6). When 20 µl of the seed extract was checked in IF, 67.3-71.3% of the samples tested were both positive or negative with dilution-plating and IF. IF negative and dilution-plating positive samples were found for 0.0-7.3% of all samples tested. The percentage of IF positive and dilution-plating negative samples ranged from 26.7-29.2 (20 µl seed extract checked) to 41.8-47.3% (50 µl seed extract checked). Generally, the probability of isolating Xcc increased with increasing numbers of fluorescent cells found in IF. Above 10 000 cells per ml the probability of isolating Xcc ranged from 57.1-81.8%. Increasing the extraction time from 5 min to 2.5 h shaking showed no significant increase of the number of samples found positive in IF and dilution-plating. However, when using both 5 min and 2.5 h shaking as compared to 5 min shaking only, more samples can be found positive in IF (1.0-14.5%) and dilution-plating (3.0-18.5 %). Examining 1 µl instead of 50 µl of the sample smear, would increase the correspondence between IF and dilution-plating results up to minimally 69.1% (MCA 20H6). However, the risk of false-negative results in IF as compared to dilution-plating would also increase.

Additional keywords: correlation, test evaluation, extraction method, media, serology, seed-borne bacteria, *Pseudomonas syringae* pv. *phaseolicola*.

Introduction

In many laboratories immunofluorescence microscopy (IF) or dilution-plating are both used to detect seed-borne bacteria (Franken and Van Vuurde, 1990). However, little is known about the correlation between results obtained by both techniques. So far, only for *Pseudomonas syringae* pv. *phaseolicola* (Psp, the causal agent of halo blight) in bean seeds and *Xanthomonas campestris* pv. *campestris* (Xcc, the causal agent of black rot) in crucifer seeds, an analysis of the correspondence and discrep-

ancy between results obtained by both tests has been made to a certain extent (respectively Van Vuurde et al. (1991) and Schaad (1982, 1983b)). Van Vuurde et al. (1991), using polyclonal antibodies in IF, compared test data of 710 samples and showed that IF was able to indicate the potential risk of halo blight for bean seed lots which were negative in dilution-plating. They demonstrated that the probability of isolating Psp increases with increasing numbers of fluorescent cells in IF. A 'grey' area of discrepancy between IF and dilution-plating was found for 17.6% of all seed samples tested. In their case the grey area included the IF positive and dilution-plating negative samples. IF negative samples were never found positive in dilution-plating in earlier experiments (Van Vuurde et al., 1983). Schaad and Donaldson (1980), using polyclonal antibodies in IF, found a grey area of discrepancy between IF and dilution-plating for 1.5% (n = 273) and 3.3% (n = 121) of the crucifer seed lots tested for Xcc in two separate experiments. The grey area included dilution-plating positive and IF negative samples as well as dilution-plating negative and IF positive samples. Schaad and Donaldson (1980) recommended that Xcc should be detected in crucifer seeds by assaying with dilution-plating and IF staining tests. Schaad (1982) found a grey area for 17.2% of all seed lots tested for Xcc. This grey area included dilution-plating positive and IF negative samples as well as dilution-plating negative and IF positive samples. Schaad (1983b) found a grey area of discrepancy of 50-60%, although only just 10 samples were tested. Schaad (1982, 1983b) using polyclonal antibodies in IF, dissuaded IF for seed certification because of lack of correlation between IF and dilution-plating on one hand, and IF and field incidence of black rot on the other hand. However, a detailed analysis of the relation between the number of fluorescent cells found in IF and the probability of isolating Xcc was not made.

The aim of the present study was to analyse in more detail the correlation between IF, using monoclonal and polyclonal antibodies, and dilution-plating for detecting Xcc in crucifer seeds.

Materials and methods

Seed lots. Naturally contaminated and 'healthy' seed lots were obtained from different parts of the world. Crucifer seed lots with low to high Xcc contamination levels (known from earlier tests and field trials) were chosen for Experiment 1. Seed lots from various origins which in earlier tests gave variable results in plating assays on NSCAA (see below) or which had high saprophyte numbers were chosen for Experiment 2. For Experiment 3 trade seed lots were tested in a seed certification program without any foreknowledge of the level of Xcc contamination or the origin of the seed lot.

Extraction method. Xcc was extracted from seed lots as described by Franken et al. (1991). In short, each cabbage seed lot tested for Xcc consisted of 10 000 or 2000 seeds. Per 1000 seeds, 10 ml of saline (0.85% NaCl) was added and seed lots were shaken for 5 min or 2.5 h at room temperature. After 5 min or 2.5 h shaking, liquid was taken for IF as well as dilution-plating.

Plating. Plating for Xcc was done on a nutrient starch cycloheximide agar (NSCA; Schaad and Donaldson, 1980) and a nutrient starch cycloheximide agar with the addi-

tion of nitrofurantoin and vancomycin (NSCAA; Randhawa and Schaad, 1984), with some modifications described by Franken et al. (1991). Suspected colonies were tested for their identity on yeast-extract- dextrose-calcium carbonate agar (YDC) and in a pathogenicity test (Schaad, 1989).

Antibodies and IF. Monoclonal antibody 20H6 (MCA 20H6) and polyclonal antiserum 94 (PCA 94) were used in IF for detecting Xcc. PCA 94 and MCA 20H6 were described and tested for specificity by Franken et al. (1992). Indirect IF was done as described by Van Vuurde et al. (1983). All IF-slides were examined by epifluorescence microscopy using a 500-1000× magnification, a field coefficient (*i*) of 18 and a tube coefficient (*R* or *K*) of 1.00-1.25. Fluorescein-isothiocyanate (FITC) was used for IF as a fluorochrome.

Statistical analysis of the data. Analysis of variance was done using the statistical program Genstat (Rothamsted Experimental Station). χ^2-tests were used for testing differences between frequencies as described by Siegel (1956). All effects and differences were tested for significance at 95 % probability level.

Results

IF and dilution-plating were both used to index seed lots for presence of Xcc. The samples tested were classified as found positive (infested) or negative (not infested) by IF and dilution-plating. The results are summarized in Table 1. When using MCA 20H6 in Experiment 1 (50 μl examined), 2 (50 μl examined) and 3 (20 μl examined), respectively 52.8%- 56.4%, 52.7% and 67.3-71.3% of all seed samples were found both positive or both negative in dilution-plating and IF. Testing the same samples with PCA 94 in Experiment 1, the results obtained with IF and dilution-plating corresponded for 45.4-56.4%. When using MCA 20H6 in Experiment 1, 2 and 3, 0.0-1.9% of all samples tested was found to be negative in IF but positive in dilution-plating. With PCA 94 a percentage of 1.8-7.3 was found in Experiment 1. In general, MCA 20H6 and PCA 94 did not differ with respect to cell counts in IF. Cell counts were, however, depended on the sample tested ($P < 0.05$). A great part of the samples tested in Experiment 1 and 2 were IF positive and dilution-plating negative. In Experiment 3 a great part of the samples tested were IF negative and dilution-plating negative.

In Experiment 1 and 3 two extraction methods were compared, viz. 5 min and 2.5 h shaking. None of the differences were significant, as tested by a χ^2-test.

A more detailed analysis was made of the relation between IF and plating results. The results presented in Table 2 show the number of positive seed samples in dilution-plating in relation to the number of fluorescent cells per ml in IF. Table 2 shows that generally more samples were found positive in dilution-plating when more fluorescent cells were found in IF. For example, when using MCA 20H6 and 2.5 h shaking in Experiment 1 23.5% of the samples classified in IF-class 1.3-2.0 were positive in dilution-plating. Of the samples classified in IF-class 4-6, 70.0% was found to be positive in dilution-plating. Some exceptional results were found, e.g. in Experiment 3 more samples were found positive in dilution-plating in IF- class 1.7-2.0 than in IF-class 2.0-4.0. The coefficient of correlation *r* between log cells per ml in IF and log

Table 1. Comparison of immunofluorescence microscopy (IF) and dilution-plating for detecting *Xanthomonas campestris* pv. *campestris* in crucifer seeds.

Study[1]	Extraction method	Number of positive samples in dilution-plating		Number of negative samples in dilution-plating		Percentage of both IF and dilution-plating positive or negative samples
		IF +	IF −	IF +	IF −	
Experiment 1						
PCA 94	5 min shaking	17 (30.9%)	4 (7.3%)	26 (47.3%)	8 (14.5%)	45.4%
	2.5 h shaking	24 (43.7%)	1 (1.8%)	23 (41.8%)	7 (12.7%)	56.4%
MCA 20H6	5 min shaking	20 (36.4%)	1 (1.8%)	25 (45.4%)	9 (16.4%)	52.8%
	2.5 h shaking	24 (43.7%)	1 (1.8%)	23 (41.8%)	7 (12.7%)	56.4%
Experiment 2						
MCA 20H6	5 min shaking	26 (13.9%)	3 (1.6%)	86 (45.7%)	73 (38.8%)	52.7%
Experiment 3						
MCA 20H6	5 min shaking	18 (17.8%)	0 (0.0%)	33 (32.7%)	50 (49.5%)	67.3%
	2.5 h shaking	13 (12.9%)	2 (1.9%)	27 (26.7%)	59 (58.4%)	71.3%

[1] In Experiment 1 and 2 50 μl of the sample was examined in IF; in Experiment 3 20 μl.

Table 2. The probability of isolating *Xanthomonas campestris* pv. *campestris* on NSCA and NSCAA in different classes with increasing numbers of fluorescent cells found in immunofluorescence microscopy (IF).

Experiment no.	Antibody	Extraction method	Number of samples positive in dilution-plating/Number of samples tested per IF class, based on 50 µl smear									
			< 1.3[1]		1.3-2.0		2.0-4.0		4.0-6.0		> 6.0	
1	PCA 94	5 min shaking	4/12	(33.3)[2]	4/15	(26.7)	4/13	(30.8)	9/15	(60.0)	0/0	(–)
		2.5 h shaking	1/8	(12.5)	0/5	(0.0)	8/20	(40.0)	15/21	(71.4)	1/1	(100.0)
	MCA 20H6	5 min shaking	1/10	(10.0)	2/14	(14.3)	8/19	(42.1)	9/11	(81.8)	1/1	(100.0)
		2.5 h shaking	1/8	(12.5)	4/17	(23.5)	6/10	(60.0)	14/20	(70.0)	0/0	(–)
2	MCA 20H6	5 min shaking	3/76	(3.9)	4/39	(10.3)	9/53	(16.7)	12/19	(63.1)	1/1	(100.0)
			Number of samples positive in dilution-plating/Number of samples tested per IF class, based on 20 µl smear									
			< 1.7[1]		1.7-2.0		2.0-4.0		4.0-6.0		> 6.0	
3	MCA 20H6	5 min shaking	0/50	(0.0)	5/16	(31.2)	1/20	(5.0)	12/15	(80.0)	0/0	(–)
		2.5 h shaking	2/61	(3.3)	3/8	(37.5)	2/17	(11.8)	8/14	(57.1)	0/1	(0.0)

[1] IF-classes are expressed as logarithms to base 10 of the number of fluorescent cells per ml in IF; < 1.3 and < 1.7 equals to a contamination less than 20 (50 µl of the sample smear examined) or 50 (20 µl of the sample smear examined) cells per ml, respectively ('IF negative' samples).

[2] Between brackets: the percentage of positive samples per IF-class.

Table 3. Comparison of immunofluorescence microscopy (IF) and dilution-plating results from several studies with regard to the detection of *Xanthomonas campestris* pv. *campestris* in crucifer seeds.

Study	Extraction method	Volume of smear examined in IF	Percentage of samples that gave corresponding results in both IF and dilution-plating (both assays positive or negative)	Calculated percentage[1] of both IF and dilution-plating positive or negative samples, when 1 µl of sample smear would have been examined	Calculated percentage[1] of both IF negative and dilution-plating positive samples, when 1 µl of sample smear would have been examined
Experiment 1, MCA 20H6	5 min	50 µl	52.8% (n = 55)	> 70.9%	> 5.5%
	2.5 h	50 µl	56.4% (n = 55)	> 72.7%	> 9.1%
Experiment 2, MCA 20H6	5 min	50 µl	52.7% (n = 188)	> 69.1%	> 3.7%
Experiment 3, MCA 20H6	5 min	20 µl	67.3% (n = 101)	> 73.3%	> 5.0%
	2.5 h	20 µl	71.3% (n = 101)	> 73.3%	> 5.0%
Other studies					
Schaad and Donaldson (1980)	1 min	1 µl	96.7% (n = 121)	96.7%	3.3%
			98.5% (n = 273)	98.5%	0.4%
Schaad (1982)	not stated[2]	not stated[2]	82.8% (n = 423)	unknown[2]	unknown[2] (5.4%)[3]
Schaad (1983b)	1 min	1 µl	40% (n = 10)	40%	0.0%
			50% (n = 12)	50%	0.0%

[1] Calculated by regarding samples classified in IF-class 1.3-2.0 and 1.7-2.0 (Table 2) as 'IF negative'.
[2] No detailed description of the IF-procedure was given.
[3] Between brackets: the percentage of IF negative and dilution-plating positive samples given by the reference.

cfu per ml of Xcc in dilution-plating was always within the range of 0.5-0.6.

In general, no differences were found between PCA 94 and MCA 20H6 in the distribution of the percentage of plating-positive samples over the IF- classes in Experiment 1 ($P = 0.234$). Also no significant differences were found between 5 min and 2.5 h shaking in Experiment 1 and 3 with respect to the percentage of positive isolations in the IF-classes ($P = 0.621$).

Most of the cabbage seed samples tested in Experiment 1 were classified in the IF-classes (log fluorescent cells per ml) ranging from 2.0-4.0 and 4.0-6.0. In Experiment 2 most samples were classified in the IF-class ranging from < 1.3 to 2.0-4.0. In Experiment 3 most samples were classified in the IF-class < 1.7. A relatively high percentage (12.5- 33.3%) of the samples classified in the IF-class < 1.3 in Experiment 1 was dilution-plating positive whereas in Experiment 2 and 3 the percentages were relatively low (3.9 and 0.0-3.3%, respectively).

Discussion

In the present study IF and dilution-plating were used to index seed lots for presence of Xcc. IF had the same characteristics in this study (for Xcc) as for other seed-borne bacteria such as Psp (Van Vuurde et al., 1991). More samples were found positive in IF than in dilution-plating for MCA 20H6 as well as PCA 94 (Table 1). Also Schaad (1982, 1983a, 1983b) reported for Xcc that IF could find more seed lots positive than dilution- plating. Several explanations for these results can be given. First, IF allows to detect fluorescent cells in undiluted extracts amongst high numbers of saprophytes whereas in dilution-plating a ten or hundred times dilution from the extract is sometimes needed to reduce the interference of saprophytes. Second, dead or weak cells may be detected in IF which cannot be detected in dilution-plating. Third, cross-reactions may have caused false-positive reactions in IF. However, the occurrence of cross-reactions is less likely, since a specificity-tested antiserum was used in the present study (Franken et al., 1992; Franken, 1992) and other studies cited here (Schaad and Donaldson, 1980; Schaad, 1983a, 1983b). No cross-reacting bacteria were isolated in this study. Fourth, seed lots may have contained chemical substances produced by the seeds or other micro-organisms which may have prevented growth of the pathogen.

In spite of the high sensitivity of IF, 0-7.3% of all samples were found to be IF negative and dilution-plating positive in this study (Table 1). Schaad and Donaldson (1980) and Schaad (1982) found respectively 0.4%-5.4% of all seed lots to be both IF negative and dilution-plating positive (Table 3).

The correlation between IF and dilution-plating for detecting Xcc in crucifer seed lots was compared with results reported by Schaad and Donaldson (1980) and Schaad (1982, 1983a, 1983b) (Table 3). The correlation between IF and dilution-plating was less for the seed lots tested in this study than for seed lots tested by Schaad and Donaldson (1980) and Schaad (1982). This could be due to differences in the volume of the sample smear examined. Schaad (1983a, 1983b) refers to Schaad and Donaldson (1980), who examined 1 μl. This means that the maximum sensitivity would be 1000 fluorescent cells per ml. In Experiment 1 and 2 (50 μl of the sample smear examined) the maximum sensitivity would be 20 fluorescent cells per ml. Therefore, using the data mentioned in Table 2 for calculating Table 3, this would mean that the IF positive samples given in IF-class 1.3-2.0 and a part of IF-class 2-4 (Table 2) would not have

been found IF-positive by the method used by Schaad and Donaldson (1980). Table 3 shows that the correlation between IF and dilution-plating would be higher if 1 µl (minimally 69.1% correspondence between IF and dilution-plating results) instead of 50 µl (52.7-56.4% correspondence between IF and dilution-plating results) of the bacterial smear would have been examined (Table 3). This approximates to the data given by Schaad (1982) and Schaad and Donaldson (1980) and is supported by the fact that in Experiment 3, in which 20 µl was examined, a higher correlation (67.3-71.3%) between IF and dilution-plating was found. By checking 1 µl instead of 50 µl, the detection threshold of IF will decrease and approximate to the detection threshold of dilution-plating. However, in that case the percentage of IF negative and dilution-plating positive samples will increase up to a level above 3.7% (Table 3). The fact that Schaad (1983b) observed a relatively poor correlation between IF and dilution-plating may have been due to the small number of seed lots tested.

The differences between results obtained in this study and the studies by Schaad (1982, 1983b) and Schaad and Donaldson (1980), may also in part be explained by the possibility that seed lots differed with respect to the levels of saprophytes and treatments (e.g. storage) undergone prior to seed testing. Also different extraction methods have been used. However, Schaad (1989) stated that short washes at 25 °C are generally satisfactory to isolate the pathogen from the seed.

The comparison of 5 min and 2.5 h shaking showed no differences in number of samples found positive in IF and dilution-plating (Table 1). This confirms the data presented by Franken et al. (1991). Also the percentage of positive isolations per IF-class did not differ between 5 min and 2.5 h shaking (Table 2). However, using both 5 min and 2.5 h shaking of seed lots instead of 5 min shaking only would have resulted in finding more samples infested. In this study, an extra 3.0-18.5% of the samples tested would have been found positive in dilution-plating when both 5 min and 2.5 h shaking would have been used instead of 5 min shaking only (data not shown). In IF, an extra 1.0%-14.5% of the samples tested would have been found positive when both 5 min and 2.5 h shaking would have been used instead of 5 min shaking only (data not shown). This suggests that the use of two extraction methods enhances the probability of detecting Xcc in various crucifer seed lots.

To improve the correlation between IF and dilution-plating, the plating assays may be improved by using more selective media with a high recovery of the target bacterium, such as CS20ABN medium (Chang et al., 1991). This medium was reported to detect more infested seed lots than the NSCA, NSCAA and FS medium.

To decide which test (IF or dilution-plating) is preferable for detecting Xcc in seed lots some aspects of the disease and the test should be considered. When the disease is considered to be a disease with a quarantine status, generally, a prediction of 'health' is required. When a certain tolerance for a disease is accepted, a prediction of 'disease' is required. Data derived directly or calculated from the reports by Van Vuurde et al. (1991) for Psp and Schaad (1983b) for Xcc show that IF has a relatively high predictive value of a negative result ('health'). Dilution-plating has a relatively high predictive value of a positive result ('disease'). When a prediction of 'health' of a seed lot is required, IF would give a better prediction than dilution-plating. When a prediction of 'disease' is required, dilution-plating would give a better prediction than IF. Thus, when dilution-plating positive results are obtained, the seed lot should be regarded as 'being infested'. When both IF and dilution-plating negative results are found, the

seed lot should be regarded as 'being healthy' or infested at a level below the tolerance level. When dilution-plating negative results and IF positive results are found, 'risk classes' should be used based on the number of saprophytes isolated on the plates, the number of fluorescent cells found in IF, and field studies under optimum 'disease conditions'. Such a classification scheme is now used for indexing bean seeds for Psp in the Netherlands (J.W.L. van Vuurde, unpublished).

It should, however, be noted that, as reported by Sheppard et al. (1986), the predictive values of a positive result and negative result vary with the prevalence of the disease (defined here as the amount of infested seeds per seed lot). At a low prevalence of a disease, given a specified diagnostic sensitivity and specificity, the percentage of 'false-positive' results will be much higher than at a high prevalence of the disease.

Acknowledgements

The author is grateful to Prof. Dr Ir J. Dekker, Drs R. Bino and C.J. Langerak, and Mrs P.S. van der Zouwen for critically reading the manuscript. Thanks are due to Ir J. van Bilsen, Bejo Seeds B.V. and M. Hooftman, Nederlandse Algemene Keuringsdienst voor Siergewassen (NAKS), for providing data for comparison and for comments on the manuscript.

References

Chang, C.J., Donaldson, D., Crowley, M. & Pinnow, D., 1991. A new semiselective medium for the isolation of *Xanthomonas campestris* pv. *campestris* from crucifer seeds. Phytopathology 81: 449-453.

Franken, A.A.J.M., 1992. Application of polyclonal and monoclonal antibodies for the detection of *Xanthomonas campestris* pv. *campestris* in crucifer seeds using immunofluorescence microscopy. Netherlands Journal of Plant Pathology 98: 95-106.

Franken, A.A.J.M. & Van Vuurde, J.W.L., 1990. Problems and new approaches in the use of serology for seed-borne bacteria. Seed Science and Technology 18: 415-426.

Franken, A.A.J.M., Zilverentant, J.F., Boonekamp, P.M. & Schots, A., 1992. Specificity of polyclonal and monoclonal antibodies for the identification of *Xanthomonas campestris* pv. *campestris*. Netherlands Journal of Plant Pathology 98: 81-94.

Franken, A.A.J.M., Van Zeijl, C., Van Bilsen, J.G.P.M., Neuvel, A., De Vogel, R., Van Wingerden, Y., Birnbaum, Y., Van Hateren, J. & Van der Zouwen, P.S., 1991. Evaluation of a plating assay for *Xanthomonas campestris* pv. *campestris*. Seed Science and Technology 19: 215-226.

Randhawa, P.S. & Schaad, N.W., 1984. Selective isolation of *Xanthomonas campestris* pv. *campestris*. Phytopathology 74: 268-272.

Schaad, N.W., 1982. Detection of bacterial plant pathogens. Plant Disease 66: 885-890.

Schaad, N.W., 1983a. Detection of *Xanthomonas campestris* pv. *campestris* in crucifer seeds. Seed Science and Technology 11: 573-578.

Schaad, N.W., 1983b. Correlation of laboratory assays for seedborne bacteria with disease development. Seed Science and Technology 11: 877-883.

Schaad, N.W., 1989. Detection of *Xanthomonas campestris* pv. *campestris* in crucifers. In: A.W. Saettler, N.W. Schaad & D.A. Roth (Eds), Detection of Bacteria in Seed and other Planting Material. The American Phytopathological Society, St. Paul, Minnesota, p. 68-75.

Schaad, N.W. & Donaldson, R.C., 1980. Comparison of two methods for detection of *Xanthomonas campestris* in infected crucifer seeds. Seed Science and Technology 8: 383-391.

Sheppard, J.W., Wright, P.F. & DeSavigny, D.H., 1986. Methods for the evaluation of EIA tests for use in the detection of seed-borne diseases. Seed Science and Technology 14: 49-59.

Siegel, S., 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Company, Inc., Tokyo, Japan, 312 pp.

Van Vuurde, J.W.L., Franken, A.A.J.M., Birnbaum, Y. & Jochems, G., 1991. Characteristics of immunofluorescence microscopy and of dilution-plating to detect *Pseudomonas syringae* pv. *phaseolicola* in bean seed lots and for risk assessment of field incidence of halo blight. Netherlands Journal of Plant Pathology 97: 233-244.

Van Vuurde, J.W.L., Van den Bovenkamp, G.W. & Birnbaum, Y., 1983. Immunofluorescence microscopy and enzyme-linked immunosorbent assay as potential routine tests for the detection of *Pseudomonas phaseolicola* and *Xanthomonas phaseoli* in bean seeds. Seed Science and Technology 11: 547- 559.