

Detection of *Xanthomonas translucens* pv. *translucens* in Barley Seed—A Comparative Evaluation of Techniques

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Availability of a quick, precise, yet reliable technique of pathogen detection from the seed is important for management of seedborne diseases. Barley stripe and black chaff disease caused by *Xanthomonas translucens* pv. *translucens* (Jones *et al.*) Dye is a worldwide problem affecting cereals. Recently it has also been reported as an upcoming disease of barley (*Hordeum vulgare* L.) around Jaipur in Rajasthan [1, 2]. During the present study, different test methods were compared and evaluated to identify the appropriate methodology for the detection of the bacterium and findings are presented in this communication.

The experimental seed material to study detection of the pathogenic bacterium consisted of two barley cvs., namely RD-2052 and RD-2035 collected from the healthy and diseased crops under natural field conditions. To suppress the mycoflora, the test seed material was dressed with fungitoxicant carbendazim (bavistin 50 WP) @ 1.0g kg⁻¹ prior to test to avoid interference of resident mycoflora. The presence of the bacterium in the seed material was determined by dry seed examination, rolled paper towel test, seedling symptomatology (growing on test), host plant inoculation and incubation methods viz; agar plate method, bacteriophage-plaque method and component plating method were used as recommended by Neergaard [3] and ISTA [4].

(1) Observation of dry seeds

The seeds were examined visually as well as

under stereobinocular microscope revealed the presence of characteristic brown discoloration in the form of stripes. Discolored seeds when cut in a drop of water revealed consistent bacterial ooze form stripes under microscope. Examination of dry seeds revealed that severely infected seeds could be identified visually due to presence of stripe discoloration or blemishes.

(2) Rolled paper towel test

The paper towel method revealed 55.00 and 47.36 per cent (RD-2052) and 52.05 and 45.17 per cent (RD-2035) seedling infection respectively due to *Xanthomonas translucens* pv. *translucens* and confirmed the effectiveness of the method. Singh and Rao [5] used this method and could detect *Xanthomonas oryzae* in 21 per cent rice seedlings.

(3) Seedling symptom test

The seedling symptom test gave the highest recovery of the pathogen from seed. Parashar and Leben [6] detected 83.30 per cent infection with *Xanthomonas axonopodis* pv. *cyamopsidis* in clusterbean and Chopra *et al.* [7] while detecting *Pseudomonas syringae* pv. *syringae* by this method concluded that "wounded grow on test" was better in detecting even 0.1 per cent infestation in the seeds of pea in comparison to traditional grow on test.

(4) Host plant inoculation method

In this method the suspension of bacterial cells

prepared from seeds separately on inoculation by direct spray or infiltration into a leaf whorl of seedlings could result in 16.00, 38.00 and 12.00, 32.00 (RD-2052) and 12.00, 32.00 and 08.00, 22.00 (RD-2035) per cent infection during 2001-02 and 2002-03 respectively (Table 1). The inoculation of bacterium by infiltration proved better as compared to spray inoculation. Mehta [8] has documented

the detection of *Xanthomonas campestris* pv. *undulosa* in wheat and *Xanthomonas campestris* pv. *hordei* in barley similarly and by Kennedy [9] *Pseudomonas syringae* pv. *glycinea* in soybean also.

(5) Incubation methods

(i) Agar plate method (XTS agar medium): As depicted (Table 1) during both the years of study, the

Table 1. Detection of *Xanthomonas translucens* pv. *translucens* in barley seed by different methods

Methods	RD-2052		RD-2035	
	2001-02	2002-03	2001-02	2002-03
Examination of dry seed				
(a) Black chaff symptom (%)	89.25	81.00	63.00	58.50
(b) Isolation/Ooze test	+	+	+	+
Paper towel method				
(a) Germination (%)	90.00	90.25	91.25	90.75
(b) Seedling infection* (%)	55.00	47.36	52.05	45.17
*Based on seedlings emerged				
Seed per towel = 50; Replication = 8				
Seedling symptomatology test				
(a) Germination (%)	90.00	92.00	92.00	93.00
(b) Seedling infection** (%)	63.33	58.15	59.23	54.83
**Seed per tray = 50; Replication = 4				
Agar plate method (XTS agar)				
Seedling infection*** (%)				
(a) Surface sterilized	38.00	35.00	32.00	28.00
(b) Surface unsterilized	68.00	60.00	56.00	54.00
***Seed per plate = 10; Repl ⁿ = 5				
Host plant inoculation method				
Seedling infection				
(a) Spray	16.00	12.00	17.00	08.00
(b) Infiltration	38.00	32.00	32.00	22.00
Bacteriophage - plaque method	Nil	Nil	Nil	Nil
Component plating				
(a) Surface sterilized (%)				
Husk	38.00	30.00	32.00	28.00
Pericarp (seed coat)	22.00	20.00	20.00	14.00
Endosperm	0.00	0.00	0.00	0.00
Embryo	0.00	0.00	0.00	0.00
(b) Surface unsterilized (%)				
Husk	66.00	58.00	58.00	54.00
Pericarp (seed coat)	28.00	26.00	24.00	22.00
Endosperm	0.00	0.00	0.00	0.00
Embryo	0.00	0.00	0.00	0.00

detection frequency of pathogen was more from seeds without surface sterilization and seeds with clearly visible blemishes (black chaff symptom) by unaided eye harboured the pathogen more. The use of XTS agar medium used in present study confirmed the findings of Schaad and Forster [10] who developed this method for isolating *Xanthomonas campestris* pv. *translucens* from wheat seeds and could obtain the pathogen from 15 out of 19 randomly collected commercial wheat seed lots as compared to only 3 of the 19 lots assayed on nutrient glucose agar medium (NA). Present findings thus validates the efficacy of XTS agar medium [10] which can be used for black chaff certification and quarantine purposes. These results are also in agreement with the findings of Gitatis *et al.* [11] and Dhanvantri and Brown [12] who detected *Xanthomonas campestris* pv. *phaseoli* and *Xanthomonas campestris* pv. *vesicatoria* respectively using selective agar media. However, the disadvantage of this method as pointed out by Forster and Schaad [13] themselves that only the number of viable cells of the pathogen washed from seed can be detected and not the number of contaminated seeds.

(ii) *Bacteriophage-plaque method* : The isolation of bacteriophage from infected seeds was made following the technique of Sutton and Katznelson [14]. However, no bacteriophage could be detected during both the years i.e. 2001-02 and 2002-03.

(iii) *Component plating method* : Under component plating of different seed parts the bacterium detected was maximum in husk followed by seed-coat (pericarp) and it could not be detected from the endosperm and embryo respectively in agreement to Karwasra *et al.* [15] who could not detect *Xanthomonas axonopodis* pv. *cyamopsisidis* in clusterbean from embryo and endosperm as it was located in the seed-coat. From the present study, thus of all the methods employed it could be inferred that seedling symptom test followed by XTS agar method are more suitable and yielded maximum per cent recovery of the bacterium.

REFERENCES

1. MAHARSHI, R.P. (1996). Bacterial Stripe and Black Chaff disease of Barley by *Xanthomonas campestris* pv. *translucens* : A report on the natural occurrence in Rajasthan, India. Proc. 9th Int. Conf. Pl. Pathogenic Bacteria, Madras, India, pp. 367-370.
2. MAHARSHI, R.P. (2002). Bacterial stripe and black chaff of barley in Rajasthan -A disease left mistaken for Helminthosporiosis hence properly appreciated hitherto. Paper presented at the Asian Congress of Mycology and Plant Pathology on Plant Health for Food Security, October 1-4, 2002, Mysore, Karnataka, India. (Abstr.).
3. NEERGAARD, P. (1979). *Seed pathology*. Vol. I. The MacMillan Press Ltd., London, England, pp. 203-205.
4. ANONYMOUS (1999). International Rules for Seed Testing. *Seed Sci. & Technol.*, 27, Supplement, 333 PP.
5. SINGH, R.A. & M.H.S. RAO (1977). A simple technique for detecting *Xanthomonas oryzae* in rice seeds. *Seed Sci. & Technol.*, 5: 123-127.
6. PARASHAR, R.P. & C. LEBEN (1972). Detection of *Pseudomonas glycinea* in soybean seed lots. *Phytopathology*, 62: 1075-1077.
7. CHOPRA, R., J.K. JINDAL & K.R. SHYAM (1993). Detection of *Pseudomonas syringae* pv. *syringae* in pea seed. *Indian Phytopath.*, 46: 29-32.
8. MEHTA, Y.R. (1990). Management of *Xanthomonas campestris* pv. *undulosa* and *hordei* through cereal seed testing. *Seed Sci. & Technol.*, 18: 467-476.
9. KENNEDY, D. (1969). Estimating losses caused by bacteria. In: *Methods in Phytobacteriology*, Ch. 1:9. Klement, Z. Rudolph. K. and Sands, D.C. eds. Akademiai Kiado, Budapest, Hungary, pp. 297-300.
10. SCHAAD, N.W. & R.L. FORSTER (1985). A semi-selective agar medium for isolating *Xanthomonas campestris* pv. *translucens* from wheat seeds. *Phytopathology*, 75: 260-263.
11. GITATIS, R.D., C.T. CHANG, K. SIGAM & C.C. DAWLER (1991). A differential medium for semiselective isolation of *Xanthomonas campestris* pv. *vesicatoria* and other cellulolytic *Xanthomonas* from various natural sources. *Plant Dis.*, 75: 1274-1278.
12. DHANVANTARI, B.N. & J.R. BROWN (1993). YSSM-XP medium for *Xanthomonas campestris* pv. *phaseoli*. *Can. J. Pl. Pathol.*, 15(3): 168-174.
13. FORSTER, R.L. & N.W. SCHAAD (1988). Control of black chaff of wheat with seed treatment and a foundation seed health program. *Plant Dis.*, 72: 935-938.
14. SUTTON, M.D. & H. KATZENLOSON (1953). Isolation of bacteriaophages for detection and identification of some seed-borne pathogenic bacteria. *Can. J. Bot.*, 31: 201-205.
15. KARWASRA, S.S., R.P. PARASHAR & M.L. SAINI (1983). *Xanthomonas campestris* pv. *cyamopsisidis* in clusterbean and its control. *Veg. Sci.*, 10: 51-54.