Pak. J. Bot., 20(1): 109—112, 1988.

COMPARISON OF METHODS OF INOCULATING *XANTHOMONAS CAMPESTRIS* PV. *TRANSLUCENS* IN WHEAT CULTIVARS

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Abstract

Bacterial suspension containing 2 x 10^7 colony forming units of *Xanthomonas campestris* pv. *translucens* per ml, was used for inoculating 19 long duration, 5 medium duration and 3 short duration commercial cultivars of wheat under green house conditions. Hypodermic inoculation method was better as compared to hand rubbing, brush or spray methods in the development of disease symptoms.

Introduction

Bacterial stripe of wheat, caused by *Xanthomonas campestris* pv. *translucens* (J.J. and R) Dye continues to reduce wheat production world-wide (Smith, 1919; Bamberg, 1936; & Moffett, 1982). The bacterium infects many different cereals and grasses (Boosalis, 1952; Cunfer *et al.*, 1982; Akhtar & Aslam, 1985). Efforts to control bacterial stripe have been directed towards resistance and seed treatments. Various methods of inoculations have been used to determine resistance to *Xanthomonas campestris* pv. *phaseoli* in beans (Andrus, 1948; Schuster, 1955) but information on inoculation methods with *Xanthomonas campestris* pv. *translucens* is lacking. The objective of this investigations was to develop a simple, quick and reliable method of inoculation of different wheat cultivars with *Xanthomonas campestris* pv. *translucens*.

Material and Methods

Ninteen long duration, 5 medium and 3 short duration commerical wheat cultivars were planted in 10cm diam. plastic pots containing green house potting mixture. Ten seeds were planted per pot. A culture of *X.* c. pv. *translucens* isolated from wheat was grown on yeast extract dextrose calcium corbonate (YDC) (Schaad, 1980) at 27°C for 72h. For inoculation baterial concentration in 0.01m phosphate buffer pH 7.2 was ascertained with a spectronis 20 (Baush & Lomb) at 590 nm and adjusted to 2 x 10^7 colony forming units (cfu)/ml. The inoculation methods were as follows:

Hypodermic inoculations were carried out on 20 day old seedlings using a sterile plastic syringe. Three plants in each replicate were injected with the bacterial suspension. Inoculations were made in such a way that only few µl of inoculum were infiltrated into leaves.

Table 1. Response of wheat cultivars to *Xanthomonas campestris* pv. *translucens* with different inoculation methods.

Cultivar	Incluation methods			
	Hypodermic	Sprayer	Brush	Hand rubbing
	Average Disease Intensity			
Long Duration				
Barani-83	2.66	2.33	0.33	0.66
Khyber-79	1.66	2.00	0.33	0.66
Indus-79	2.83	2.00	0.33	0.66
ARZ	2.33	1.33	0.66	1.33
Pavon	2.66	2.00	1.33	0.66
Pak-81	3.00	2.33	0.66	0.66
Lyallpur-73	2.66	1.33	0.66	0.66
ZA-77	2.00	1.66	1.33	1.00
Barani-70	1.66	1.66	1.00	0.33
Barani-79	1.66	1.66	1.00	0.33
C-271	1.66	2.00	1.33	0.66
C-273	1.66	1.00	1.00	0.66
C-518	2.00	1.66	1.53	0.66
C-591	2.00	1.00	1.00	0.66
Chenab-70	1.66	1.00	1.00	0.66
Mexipak	1.66	1.00	1.00	1.00
Pak-70	1.66	0.66	1.00	0.66
Sarhad-82	2.00	0.66	0.66	1.00
Punjab-76	1.00	1.66	0.53	1.00
Mean	2.04a	1.53b	0.86c	0.72c
Medium Duration				
LU-26	2.00	1.53	0.66	1.53
Sandal	2.00	0.66	0.53	1.00
Punjab-81	2.00	1.66	0.66	0.33
PARI-73	1.66	1.33	0.66	1.00
Yecora	2.33	1.00	0.66	1.00
Mean	2.00a	1.20b	0.93b	0.60b
Short Duration				
Bahawalpur-79	2.00	2.00	1.33	1.33
Sonalika	1.66	1.33	1.00	1.00
T.J-83	2.00	1.00	1.00	0.66
Mean	1.89a	1.44ab	1.11b	1.00b

*Disease intensity = 0 = No disease, 1 = Lesion 5 mm (resistant), 2 = Water soaked lesion 5-15 mm in diameter (Susceptible), 3 = Water soaked lesion 7-17 mm in diameter (very susceptible).

**Disease intensity is average of nine plants.

Locally made atomizer was used for inoculating the plants. Bacterial suspension was atomized directly on to leaves to run-off point. The plants were kept in a humid chamber for 72h after inoculation.

Leaves of the wheat seedling were moistened with sterile water and bacterial suspension was then rubbed on the leaf surface with the help of a fine camel hair brush.

Wheat seedlings were washed thoroughly with sterile water then inoculated by rubbing the leaves gently between the first finger already dipped in the bacterial suspension.

After inoculation the plants were incubated in dark at 100% R.H and after 72h they were placed on a glass house bench at 20-29°C and 60-100% R.H.

Results and Discussion

Translucent elongated water soaked stripes appeared within 4 days after inoculation. Bacterial exudate was present on most of the lesions. The hypodermic method provided more uniform results, since the symptoms were visible 72h after inoculation. The brush and hand rubbing methods showed less disease production since the bacteria cannot penetrate the intact host surfaces. In screening for resistance such methods can therefore not be used with confidence. In the long duration group where hypodermic inoculation method was used the average bacterial stripe severity ranged from 1.0-3.0 in the cv. Punjab-76 and Pak-81 respectively. The cultivars Pak-70 and Barani-83 showed disease severity 0.66-2.33 with the spray method while in Khyber-79 and Pavon disease intensity ranged from 0.33-1.33 with brush method. With the hand rubbing method disease severity in Barani-70 and ARZ was 0.33 and 1.33, respectively. In the medium group, Yecora showed a disease severity 2.23, while LU-26, Sandal and Punjab-81 came up with a disease rating of 2 with the hypodermic method. In the short duration group Bahawalpur-79 showed a disease rating of 2.00 with both the hypodermic and sprayer methods, while it had a rating 1.33 with the brush and hand rubbing methods (Table 1). Although hypodermic method of inoculation was significantly different from hand rubbing and brush methods for the long and medium group, but in the short duration group, the hypodermic and sprayer methods were not significantly different from each other. The present findings are in conformity with that of Bamberg (1936), Boosalis (1952) and Cunfer *et al.*, (1982). It would suggest that hypodermic inoculation provides a reliable method for screening of germ plasm for resistance against bacterial stripe.

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(Received for publication 10 September 1987)