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Kresek Phase of Rice Bacterial Blight (*Xanthomonas Orysae*) Disease in India II. Role of Inoculum Concentration and Periods of Inoculation on the Disease *

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Studies on the effect of inoculum concentration and duration of root inoculation revealed that these factors can directly influence the severity of Kresek (wilt) phase of rice bacterial blight and inversely the incubation period in expressing the disease symptoms. A minimum inoculum pressure at 10^7 cells/ml and prolonged root exposure to inoculum (about 48 hr or above) were highly favourable for severe form of wilt appearance under water culture.

The Kresek (wilt) is a serious phase of rice bacterial blight disease (*Xanthomonas oryzae*) especially in tropical countries and may cause complete failure of the crop (Ou, 1972). In India, the wilt is known to reduce the yields more than the leaf blight phase in certain localities (AICRIP, 1971; Srivastava, 1972; Sulaiman, 1965). The roots of the rice seedling have been activating bacterial infection and developing the Kresek phase (Eamchit and Ou, 1970; IRRI 1966; Mizukami, 1957). The study in Japan showed that root inoculation of the pathoge.. resulted in abnormal growth and death of seedlings in water culture. But the mechanism by which *X. oryzae* reach vessel element and develop Kresek, the vascular wilt symptom, is a little understood phenomenon. However, Srinivasan (1980) observed the wounded roots serving as the possible path for invasion of the pathogen to cause Kresek phase. Wilt infection in rice plants can be due to the systemic bacterial build up (Watanabe, 1975). The wounded cut ends of roots may serve as the entry points to inoculum only for short period after wounding (Yamamoto, and Yoshimura, 1970). But the role of inoculum concentration and period of root exposure in development of wilt phase have not been known. The impact of inoculum pressure on disease severity have been reported for bacterial wilts caused by *Corynebacterium michiganense* (smith) Jensen (Thyr, 1968; Strider, 1970) and *Pseudomonas solanacearum* smith (Winstead and Kelman, 1952). The present

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paper reports the effect of root exposure on development of Kresek phase under water culture technique.

MATERIAL AND METHODS

Twenty five day old healthy seedlings of I R 8 rice were used in the experiment. The root system of the seedlings was wounded by "cutting off" of the root tips and surface sterilized in 70 per cent alcohol. The bacterial suspension (stock inoculum) of a virulent isolate was prepared from 72 hr old cultures and the experiments were conducted in water culture which was standardized previously (Srinivasan, 1980).

The inoculum concentrations at nine different levels of 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9 and 10^{10} cells/ml were used to find the effect of inoculum at different levels of pressure on the development of wilt phase. Twenty seedlings were inoculated for each treatment by dipping the roots in 10 ml of bacterial suspension in culture tubes (15.5 × 1.3 cm size) replicated twice. One seedling was placed in each tube. The mouth of the tube along with seedling was loosely plugged with cotton.

To find out the effect of different periods of root inoculation on the disease, another experiment was conducted. The roots of the seedlings were exposed to a standard inoculum (1×10^8 cell/ml) for 0.5, 2.0, 4.0, 6.0 12.0, 24.0, 30.0, 36.0, 48.0, 72.0, 96.0, 144.0 and 192.0 hr. Twenty seedlings were inoculated in each treatment and transferred to culture tubes having 10 ml of sterile distilled water at the rate of one seedling per tube and maintained as in the previous experiment.

The experimental seedlings with adequate controls were kept for 30 days in greenhouse with conditions favourable for the disease. The water level of the seedling tubes was adequately maintained. The development of Kresek phase was recorded at different intervals after the root dip. The data were arranged concisely and tested by suitable method.

RESULTS AND DISCUSSION

The bacterial inoculum at different concentrations influenced the period for development of Kresek symptom. The disease developed after 15 days of inoculation at the concentrations of 10^2 and 10^3 cells/ml while it was reduced to 8 days when the inoculum pressure is increased (Table I). Watanbe (1975) could not find the disease development at a lower inoculum level (10^2 cells/ml) but observed to occur when the concentration was above 10^4 cells/ml. In the present study, the development of wilt at lower inoculum itself, though the incubation period was prolonged, is suggestive of the virulence of the pathogenic isolate tested and the sensitivity of the technique adopted.

The effect of inoculum concentration in increasing the percentage of infected seedlings is more pronounced after 15 days of inoculation. The disease incidence was 100 per cent on 30th day after inoculation with 10^9 — 10^{10} cells/ml, followed by 90 per cent Kresek at 10^7 — 10^8 cells/ml. The disease intensity became less when inoculum pressure was reduced further and minimum (10 per cent) at an inoculum level of 10^2 cells/ml. The intensity of tomato wilt due to *P. solanacearum* on certain cvs. has been related to inoculum concentration (Winstead and Kelman, 1952). Strider (1970) made root inoculation of tomato seedlings with *C. michiganense* and observed severe wilt when the inoculum level was at 10^9 cells/plant but no disease was observed at 10^6 cells/plants. These results are in agreement with the present study and suggest that the concentration of inoculum to a certain extent directly influences the intensity of Kresek phase and also inversely affects the incubation period in expressing the disease symptom.

The Table II reveals that the roots exposed to inoculum for 0.5 hr failed to develop the disease. The Kresek phase made its appearance with a lag period of 20 days following the root exposure for 2.0 — 6.0 hr; but its intensity was limited to 10 per cent which remained static after the initial appearance. The wilt intensity in other treatments was to a certain extent in proportion to the duration of root exposure. The over-all effect of periods of root exposure on disease severity was significant during different periods of observation and was more pronounced on 30th day after inoculation. One hundred per cent disease intensity was observed on 30th day when roots were exposed to inoculum for 48 - 192hr. When duration of root exposure was reduced the incidence of Kresek was less on this day. Thirty per cent of disease intensity observed due to 12 hr of root exposure. The disease intensity reduced significantly when the inoculation period was limited to 6hr or less.

Yamamoto and Yoshimura (1970) viewed that the cut ends of roots can serve as the entry points to *X. oryzae*, only for short period after cutting. In the present study, the duration of root exposure to inoculum influenced the Kresek intensity and inversely affected the incubation period. A minimum "Root - Inoculum" contact period of about 2.0hr. was essential for development of the wilt symptom under the technique adopted. It appears that the prolonged root exposure to inoculum facilitates the better establishment of the pathogen within the host plant as revealed by the development of severe form of Kresek after a long period of inoculation.

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TABLE I Effect of *X. oryzae* concentration on the development of Kresek symptom at different days after inoculation.

Inoculum concentration (cells/ml)	Per cent infected seedlings at different days after inoculation					
	8	10	15	20	25	30
10^2	0	0	5	5	10	10
10^3	0	0	10	20	20	25
10^4	10	10	20	35	45	60
10^5	10	20	30	40	60	75[a]
10^6	10	30	50	75	80	80[a]
10^7	15	45	60	90	90	90
10^8	30	75	90	90	90	90
10^9	30	80	90	100	100	100
10^{10}	40	90	90	100	100	100
Control	0	0	0	0	0	0
Chi-Square value (df: 8)	51.00*	418.11*	392.97*	458.89	419.10	419.11*

a = Non-significant

* = Significant at 0.05 level of probability

TABLE II Effect of dipping the roots in *X. oryzae* suspension for different periods on the development of Kresek symptom at different days after inoculation.

Period of root exposure (hr)	Per cent infected seedlings at different days after inoculation				
	10	15	20	25	30
0.5	0	0	0	0	0
2.0	0	0	5	5	5[a]
4.0	0	0	5	5	5[a]
6.0	0	0	10	10	10[a]
12.0	5	15	15	20	30
24.0	20	40	50	55	70
30.0	40	50	60	75	85
36.0	40	65	65	80	85
42.0	50	65	75	90	100
72.0	60	75	85	95	100
96.0	75	75	85	100	100
144.0	75	85	90	100	100
192.0	75	85	100	100	100
Control	0	0	0	0	0
Chi-Square value (df: 12)	522.82*	604.09*	674.80	861.54*	939.25*

a = Non-significant
* = Significant at 0.05 level of probability