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STUDY ON KRESEK (WILT) OF THE RICE BACTERIAL BLIGHT SYNDROME

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ABSTRACT

Kresek (or wilt) symptom of the bacterial blight syndrome developed on rice seedlings when they were inoculated either by leaf clipping or by root-dipping. Kresek resulted with less frequency when older plants were infected with leaf blight. The pale yellow symptom was found to be a secondary effect of either leaf blight or kresek. The older the plants, the lower the percentage of kresek induced by root dip. There were varietal differences to kresek infection. IR1545 was as susceptible as IR8 from 9 to 16 DS, but only a minimum incidence of kresek was induced at 32 DS. Varieties such as IR20 and IR1545 are resistant to leaf blight but as susceptible as IR8 to kresek. Dipping seedling roots in zinc solution before transplanting reduced the incidence of kresek.

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STUDY ON KRESEK (WILT) OF THE RICE BACTERIAL BLIGHT SYNDROME

Bacterial blight caused by *Xanthomonas oryzae* has three symptoms: leaf blight, kresek (wilt), and pale yellow. Kresek was first reported in 1950 by Reitsma and Schure in Indonesia, and assumed to be a new disease. In 1964, Goto (1964), working at the International Rice Research Institute (IRRI), identified the causal bacterium as *X. oryzae* and confirmed that kresek is a symptom of the bacterial blight syndrome. Since then, kresek has been recognized in the Philippines (IRRI 1967), Malaysia (Singh et al 1970), India (Devadath and Dath 1970), Sri Lanka (Shigemura and Tabei 1969), and recently in China (Chou, L. K., personal communication) and Korea (Choi et al 1977). Information from China and Korea indicates that kresek is not restricted to the tropics.

Common in tropical Asia, kresek is the systemic infection caused by *X. oryzae* that generally damages rice more than leaf blight alone. It usually occurs from 1 to 6 weeks after transplanting. Kresek-infected plants wilt rapidly and either die or partially recover to produce new tillers. The leaf blight symptom may then occur. In the field, kresek resembles the stem borer damage known as *deadheart*. However, kresek can be identified by squeezing the infected stem tissues so that the bacterial mass oozes out.

Despite early works by Goto (1964) and others (Devadath and Dath 1970, Shigemura and Tabei 1969, Singh et al 1970, Tabei 1977, Watanabe 1975, Yoshimura and Iwata 1965, Yoshimura et al 1965, Yamamoto 1976), the pathological relationships between the three symptoms of the bacterial blight syndrome are not fully understood. In this paper, we report research at IRRI on kresek infection, methods of inoculation, and the relationship between varietal resistance to leaf blight and to kresek.

MATERIALS AND METHODS

Bacterial isolates and inoculum preparation

We used two strains of *X. oryzae* throughout the experiments. The leaf blight strain, designated as PXO61, was isolated in 1973 from naturally infected rice leaves and has been used in screening for leaf blight resistance ever since. PXO82, the kresek strain, was isolated from the stem tissues of a naturally infected IR20 plant. Results from the test of pathogenicity indicated that both strains cause leaf blight by leaf-clipping and kresek by root-dipping. PXO82 was later found to be a strain from virulence group 2, whereas PXO61 is from group 1. The two virulence groups are distinguished by their differential infection on rice varieties with specific resistance to leaf blight (Mew and Vera Cruz 1979). Varieties carrying *Xa4* for resistance are resistant to group 1 but susceptible to group 2.

Pure cultures of the two strains were maintained on agar slants of potato or peptone sucrose medium (PSA) at -10°C. When used, they were subcultured on the same medium. Inoculum of each strain was prepared on PSA from 72-h-old cultures. The inoculum concentration was adjusted to an optical density of 1 ca 10^9 cells/ml on a Coleman Nephocolorimeter with a 590 nm filter and designated as 10°. Serial dilutions were based on this concentration to infect rice plants with kresek at different inoculum concentrations.

Rice varieties and plant age at time of inoculation

IR8, which has no functional gene for resistance to bacterial blight in the Philippines, was used throughout the study. IR20, IR1545-339 (referred to here as IR1545), DV85, and IR1698 which carry *Xa4*, *xa5*, *xa5* + *Xa7*, and *Xa6* genes, respectively, for resistance to leaf blight were also used to compare the relationship between leaf blight resistance and kresek infection.

To evaluate varietal resistance to kresek, 14 selected varieties were tested both in the greenhouse and in the field. For both experiments seedlings were raised in seedboxes in the greenhouse. At 9 to 14 days after sowing (DS), seedlings in the greenhouse experiments were transplanted singly to seedboxes. In the field tests, seedlings were transplanted at 21 DS. At transplanting, the seedling leaf tips were usually trimmed off about 50% at 14 and 21 DS, but not at 9 DS. Both split plot and randomized complete block designs were used.

Methods of inoculation

Preliminary tests indicate that kresek can be induced through either leaf infection by the clipping method or dipping the roots in a bacterial suspension. Yamamoto (1976), in screening for bacterial blight, found the method effective for kresek. An initial study indicated that a 1-min dip was sufficient to induce 50% kresek. Subsequent experiments used a 5-min root dip. Most of the results reported here are based on the root-dip method.

The roots of rice plants pulled from the seedboxes were washed thoroughly with tap water. After trimming off the tips with scissors, the roots were dipped into one of the serial dilutions of the bacterial suspension for 5 min. The method was applied to both the greenhouse and the field experiments.

Disease measurement

The disease measurement of kresek was based on two criteria throughout the study: the rolling and wilting of newly emerged leaves or the death of whole plants.

RESULTS

Time and number of plants for kresek infection evaluation

In a preliminary greenhouse test, the percentage of kresek infection peaked at 3 to 4 weeks after root-dip inoculation. Experiments were designed to determine the number of IR8 plants necessary for studying kresek and for varietal testing.

For practical purposes, 60 to 80 plants scored from 21 to 24 days after inoculation are adequate for greenhouse experiments on kresek (Table 1). The experiments that follow are based on this scoring system unless otherwise stated.

Effect of plant age on kresek development

In the field, kresek is normally observed from 1 to 6 weeks after transplanting. It may occur in adult plants if the disease conditions are favorable or the variety is very susceptible. In experimental conditions, seedlings have more kresek than older plants.

IR8 and IR1545 were evaluated for response to kresek at different ages. The two varieties had high percentages of kresek infection from 9 to 16 DS (Fig. 1). At 23 DS, IR8 had 90% infection and IR1545 had 54 but at 32 DS, IR8 had 72% and IR1545 had only 8.

Table 1. Coefficient of variation[a] for percentage of infection based on number of plants tested (IR8) by root-dip method of inoculation. IRRI, 1977.

Days after inoculation	Coefficient of variation (%)				
	20 plants	40 plants	60 plants	80 plants	100 plants
6	171	161	143	133	89
11	67	46	43	33	30
15	21	29	22	18	17
18	26	20	20	15	14
22	26	14	16	13	12
27	20	13	13	10	9

[a]Estimated standard error of the mean.

Kresek development and leaf blight infection

Nine-day-old IR8, IR20, and IR1545 plants, inoculated with strain PX082 and greenhouse grown, had 83, 72, and 80% kresek infection, respectively. DV85, 26 days after inoculation (Fig. 2), had 49%. The difference in kresek infection among IR8, IR20, and IR1545 was not significant but the difference was significant between DV85 and the other three varieties. Although the final percentage of kresek infection in IR1545 did not differ from that in IR8 or IR20, the lag phase of kresek development was considerably lower in the former.

When the four varieties were evaluated for leaf blight at 9 and 16 DS, IR8 and IR20 were susceptible to PX082 as expected (Table 2). Both IR1545 and DV85 were moderately resistant at 9 DS, and DV85 was resistant at 16 DS.

Difference between X. oryzae strains in kresek development

Isolates from leaf blight-infected plants (LB isolates) and isolates from kresek-infected plants (Kr isolates) were compared for their capacity to produce kresek. Five isolates from each group were collected from naturally infected plants. Each isolate was capable of causing kresek and leaf blight, but the percentage of kresek-infected plants varied among the four varieties (IR8, IR20, IR1545, and IR1698) tested, and also among isolates (Table 3). Some LB isolates caused as much kresek as or more than Kr isolates. For example, on IR8, the LB isolate PX061 caused 100% kresek but Kr5 caused 85%. On the basis of overall reaction, Kr isolates produced higher percentages of kresek than the LB isolates on each of the four varieties (Table 4).

Varietal difference to kresek infection

In greenhouse experiments, DV85 exhibited less kresek than the other varieties tested. Fourteen varieties with varying resistance to leaf blight were evaluated in the greenhouse for their kresek reaction to strains PX082 and PX061, and in the field for reaction to PX082. All except IR8, India Dular, Ketan Lumbu, and Lakshmi Digha varied in their reaction to the two strains in the greenhouse (Table 5). India Dular showed the least kresek infection among the 14 varieties in the greenhouse. Together with Semora Mangga, it also ranked the most resistant variety in the field (Table 6). Based on 100 hills per variety scored in the field, the fifth week after inoculation at transplanting seems the proper time for scoring.

Effect of zinc compounds on X. oryzae and kresek development

Dipping seedlings in a zinc oxide or zinc sulfate solution before transplanting is recommended and widely adapted by farmers in the Philippines to compensate for zinc-deficient soil conditions. IRRI field plots identified with high kresek incidence among seedlings no longer showed the kresek after

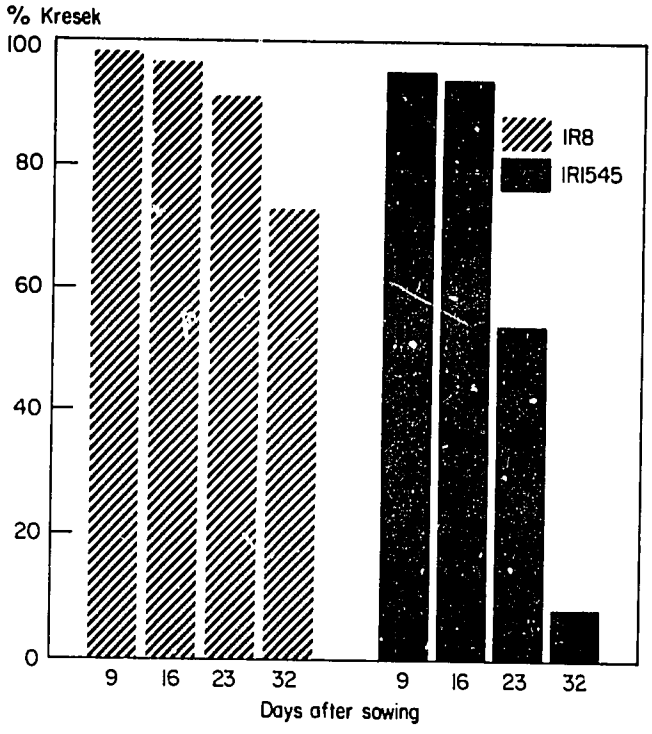

Fig. 1. Development of *kresek* at different ages of IR8 which has no gene for leaf blight resistance and IR1545 which has a recessive gene.

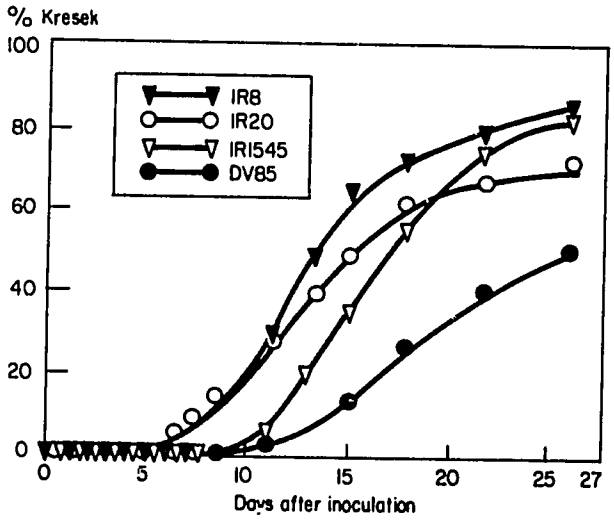

Fig. 2. Development of *kresek* induced by isolate PX082 of *X. oryzae* on 9-day-old seedlings of four rice varieties.

zinc dipping. The effect of the zinc compounds on the growth of *X. oryzae* and kresek development was therefore evaluated.

We studied the effects of zinc sulfate on the growth of *X. oryzae* in potato sucrose agar medium. Zinc sulfate applied at 2.5, 1.25, and 0.625% solutions by the paper disc method inhibited the growth of the bacterium (Table 7). The inhibition zone decreased as the concentration of zinc sulfate decreased from 2.5 to 0.625%. When different concentrations of zinc sulfate were incorporated into the potato sucrose broth medium and incubated from 0 to 96 h at 28°C, no *X. oryzae* growth was detected (Fig. 3). But the bacterium grew well in the same medium without zinc sulfate.

Two-week-old greenhouse seedlings of IR8 were root-dip inoculated with PX082, and then dipped again in a 2% $ZnSO_4$ solution before transplanting. Zinc dipping apparently controlled kresek development.

Twenty-one-day-old seedlings were root-dip inoculated with PX061 for 5 to 10 min, then dipped again in either a zinc solution or Kocide, a copper fungicide

Table 2. Reaction of four rice varieties to the infection of *kresek* and leaf blight caused by *Xanthomonas oryzae* isolate PX082.

Variety	Kresek[a]	Leaf blight[b]	
		9 DS	16 DS
IR8	83.7	1.00 (S)	1.00 (S)
IR20	72.0	1.00 (S)	0.97 (S)
IR1545	82.9	0.39 (MR)	0.34 (MR)
DV85	49.2	0.33 (MR)	0.10 (R)

[a]Percent of 100 seedlings inoculated at 9 DS, scored at 27 days after inoculation. [b]Ratio of lesion length/leaf length. DS = days after sowing, S = susceptible, MR = moderately resistant, R = resistant.

Table 3. Kresek production by leaf blight (LB) and kresek (Kr) isolates of *Xanthomonas oryzae* in four rice varieties. IRRI, 1977.

Isolate	Infection[a] (%)				Mean
	IR8	IR36	IR1545	IR1698	
LB 158	37 a	1 a	25 a	3 a	16
LB 200	90 bc	77 cd	48 ab	51 bcd	67
LB 227	94 bcd	72 cd	51 b	44 bc	65
LB 273	97 bcd	37 b	50 b	32 b	54
PX061	100 d	68 cd	67 bc	75 d	78
Kr 4	98 bcd	86 d	78 c	65 cd	82
Kr 5	85 b	78 cd	58 bc	50 bcd	68
Kr 10	91 bcd	82 cd	70 bc	50 bcd	73
Kr 55	85 b	60 bc	68 bc	45 bc	65
Kr 79	99 cd	86 d	77 c	67 cd	82

[a]In a column, means followed by a common letter are not significantly different at the 5% level.

Table 4. Difference in kresek infection from leaf blight (LB) isolates and kresek (Kr) isolates of *Xanthomonas oryzae*, and overall varietal response. IRRI, 1977.

Variety	Kresek infection (%)			Overall[b] reaction
	LB isolate	Kr isolate	Difference[a]	
IR8	84[c]	92	8 ns	88
IR36	51	79	27**	65
IR1545	48	70	22**	59
IR1698	41	56	14**	48

[a]ns = not significant, ** = significant at the 1% level. [b]Mean of LB isolates and Kr isolates. [c]Mean of kresek percentage based on five isolates in each group.

Table 5. Kresek and leaf blight reactions of selected varieties to isolates of *Xanthomonas oryzae*. IRRI, 1977.

Variety	Reaction				
	Kresek (%)		Leaf blight[a]		
	PX061	PX082	PX061	PX079	PX071
IR8	82	79	S	S	S
IR20	55	70	MR	S	MR
IR1545-339	67	92	R	R	S
DV85	34	24	R	R	R
ARC 6076	52	79	S	R	S
Bowalia	65	44	R	R	MR-MS
Buri Katari	38	26	R	R	R
Dharial	[b]	57	R	R	MR-MS
Djawa Tulen	47	35	S	S	MS-S
India Dular	14	13	R	R	R
Kasia Panja	84	62	R	MR	MR
Ketan Lumbu	21	21	[b]	[b]	[b]
Lakshmi Digha	37	31	R	R	R
Pelita I-1	78	86	MR	S	MR

[a]R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible. [b]No test results.

(cupric hydroxide), before transplanting in the field during the dry season. Kresek measured either on a tiller basis or hill basis indicated that Kocide provided better control than the $ZnSO_4$ or ZnO (Table 8). Based on natural infection, however, all three chemicals provided some control against kresek infection; Kocide and $ZnSO_4$ resulted in less kresek.

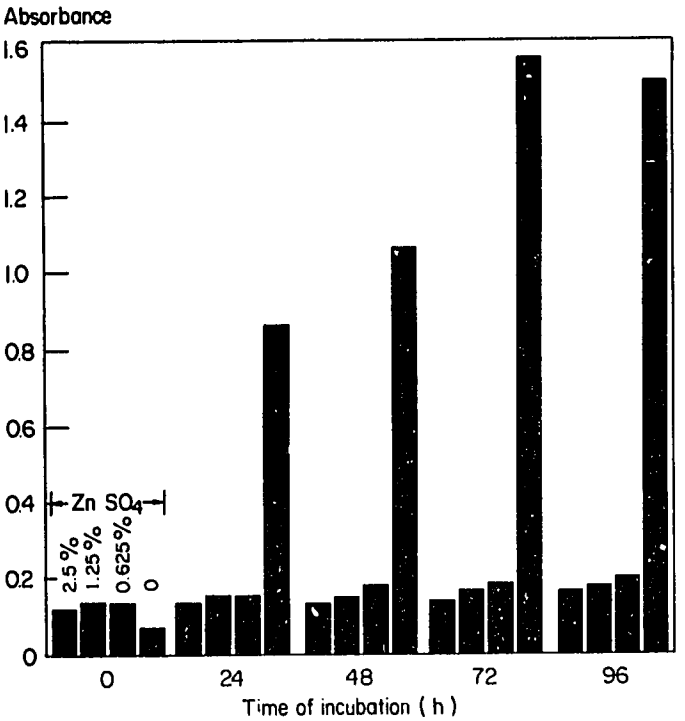

Fig. 3. The effect of $ZnSO_4$ on the growth of *Xanthomonas oryzae* by shake culture.

Measurement of the bacteriophage population by the plaque count method from water samples taken from the different plots indicated that phage populations in check plots were higher than in the chemical-treated plots.

DISCUSSION

During the present study, no inoculated plants showed the pale yellow symptom that precedes leaf blight or kresek symptoms. Inoculation resulted in leaf blight symptoms. Kresek symptoms could be developed from these plants when they were infected as seedlings. Kresek was less frequent when older plants were infected with leaf blight. The relationships between the three symptoms of the rice bacterial blight syndrome caused by *X. oryzae* are illustrated in Figure 4. Although *X. oryzae* is involved in all symptoms, kresek and leaf blight seem distinct and independent of each other. Individual plants may suffer from either kresek or leaf blight infection. This indicates that kresek and leaf blight are the primary effects of infection. Pale yellow is a secondary effect of either leaf blight or kresek, which explains why attempts to isolate the bacterium from pale yellow leaves have failed. Attempts to isolate the bacterium from leaves 1 to 2 days after kresek symptoms developed also failed.

The root-dip method of inoculation resulted in a higher percentage of kresek. Although kresek could also be initiated from leaf infection, the incidence varied and occurred only when the plants were young

Table 6. Varietal differences in kresek infection in the field (PXO82) 1 to 6 weeks after inoculation. IRRI, 1977.

Variety	Kresek[a] (%)					
	1 wk	2 wk	3 wk	4 wk	5 wk	6 wk
India Dular	1 a	1 a	4 a	7 a	10 a	10 a
Semora Mangga	0 a	1 a	4	8 a	10 a	11 ab
Kasia Panja	1 a	1 a	8 ab	14 ab	17 ab	17 abc
Bowalia	1 a	3 ab	6 ab	15 ab	18 ab	19 abc
ARC 6076	1 a	3 ab	7 ab	15 ab	20 ab	22 abcd
DV85	1 a	5 ab	11 ab	21 b	25 bc	22 abcd
Buri Katari	1 a	3 ab	9 ab	17 ab	21 abc	23 abcd
Ketan Lumbu	2 a	3 ab	8 ab	14 ab	19 ab	23 abcd
Lakshmi Digha	0 a	1 a	11 ab	17 ab	23 abc	23 abcd
Dharial	2 a	5 ab	13 ab	17 ab	22 abc	25 bcd
Djawa Tulen	2 a	6 abc	12 ab	22 b	28 bc	28 cd
IR1545	0 a	2 ab	14 ab	26 b	36 cd	37 de
Pelita I-1	2 a	6 abc	17 b	24 b	29 bc	29 cd
IR8 (check)	1 a	13 c	29 c	38 c	41 d	42 e

[a]In each column, any two means followed by a common letter are not significantly different at 5% level.

and the variety very susceptible. No kresek could be induced on plants of a resistant variety through leaf infection. Kresek appeared when resistant varieties IR20, IR1545, and DV85 were root-dipped, but kresek incidence in IR20 and IR1545 was similar to that of IR8 (Fig. 1, Table 2). IR20 was susceptible and IR1545 was resistant to leaf infection by PXO82, but there was no difference in the percentage of kresek between the two varieties when compared to IR8. At 9 and 16 DS, the leaf blight reaction of the three varieties was comparable to that at the maximum tillering stage: IR8 and IR20 were susceptible to PXO82 and IR1545 was resistant or moderately resistant. Leaf blight resistance, therefore, may not be similar to that of kresek, which could be due to the sites of primary infection: leaf blight via leaf and kresek more often via wounded roots.

Plant age seems to have a significant effect on the infection whether or not kresek results from root injury or hydathodes of leaf blades. The older the plants, the less the kresek incidence. From 9 to 16 DS, the percentage of kresek infection in IR1545 was similar to that of IR8. Kresek was reduced to 8% in IR1545 when the infection started at 32 DS; on IR8, kresek was 72% at the same age.

The kresek strain of *X. oryzae* seems to produce a higher percentage of kresek than the leaf blight strain based on the overall reaction. But there are variations among each strainal group. However, the strains show no difference in pathogenicity (Mew 1978) to leaf blight and kresek. There are also no indications from the present study that the

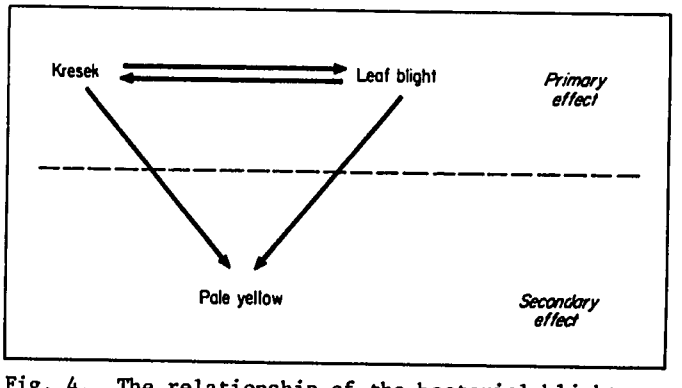

Fig. 4. The relationship of the bacterial blight syndrome.

Table 7. Effect of $ZnSO_4$ on the inhibition of *Xanthomonas oryzae* on agar plate by the paper-disc method.

Concentration of $ZnSO_4$ (%)	Inhibition zone (cm)	Growth reduction zone (cm)
2.5	1.46	1.77
1.25	1.19	1.47
0.625	0.67	1.48
CK (H_2O)	0.00	0.00

Table 8. Effectiveness of some selected chemicals on the control of kresek on IR8 in the field.

Treatment	Percent control[a]			
	Artificial inoculation[b]		Natural infection	
	Hill basis	Tiller basis	Hill basis	Tiller basis
$ZnSO_4$ (2.5%)	14.4	30.9	74.4	85.7
ZnO (2.0%)	-6.2	-15.6	59.8	74.3
Kocide (1.0%)	53.5	69.8	93.3	94.3
Check (H_2O)	0.0	0.0	0.0	0.0

[a] $\frac{\text{Treatment-check}}{\text{Treatment}} \times 100\%$. [b] Artificial inoculation by root-dip method before chemical treatment.

most virulent strain of *X. oryzae* will cause kresek and the less virulent one will cause leaf blight, as concluded by Devadath and Dath (1^70).

The results suggest that varietal response to kresek infection may be different from that to leaf blight infection. All varieties tested, except IR8, India Dular, Ketan Lumbu, and Lakshmi Digha varied in their reaction to leaf blight and kresek strains.

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