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Effects of Plant Age on Symptom Development Produced by *Pyricularia oryzae* Toxin

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ABSTRACT

The main objective of this experiment was to determine suitable plant age for toxin inoculation on detached leaves and whole plant conditions. For detached leaves inoculation, it was found that 20 day old plants were the most sensitive to both crude extract toxin (CET) derived from culture filtrate of *P. oryzae* and tenuazonic acid. The degree of sensitivity was manifested by yellowing produced around the lesions of inoculated leaves. Forty day old plants were found to be the most appropriate age for CET inoculation as they produced typical blast symptom while tenuazonic acid could produce similar typical blast symptoms on leaves at all plant ages. The reactions to CET were varied to the different plant ages while the reactions to tenuazonic acid were not significantly different among them. The experimental result indicated that the lesion sizes produced on blast susceptible and resistant varieties upon CET and tenuazonic acid were not significantly different. In general, leaves exhibited higher sensitivity to CET than tenuazonic acid. It probably might be due to the concentration of the former higher than the latter. CET at concentration of 25% was the most appropriate for spray inoculation on 2-3 week-old seedlings.

Key words: crude extract toxin, tenuazonic acid, blast, *Pyricularia oryzae*, plant ages

INTRODUCTION

There were reports that phytotoxic substances from *Pyricularia oryzae* Cav. could produce several types of symptoms and effects on rice plants. Piricularin, one of toxic substances from *P.oryzae,* produced brown necrotic spots and inhibited seedling growth (Tamari and Kaji, 1959). A novel phytotoxin, tenuazonic acid, have been reported on rice plants. Tenuazonic acid at the concentration of 50 ppm completely inhibited growth of rice seedlings and it produced typical blast symptom on rice leaves. (Umetsu *et al.*, 1972). Iwasaki *et al.* (1972) reported that tenuazonic acid at the concentration of 200 ppm inhibited shoot and root growth. It could produce small brown spot (Kozaka *et al.*, 1985) and typical blast symptom after inoculation by micropipette on detached leaves (Singburaudom *et al.*, 1995).

It is known that blast fungus penetrates into host surface through a penetration peg making mechanical injury to host surface for facilitating penetration through epidermal cells. However, there are little informations regarding the entering of toxin into host. Chaudhary *et al.* (1995) recently has reported that crude extract toxin of *P. oryzae* could produce typical blast symptom with and without mechanical wounding on rice leaves prior

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to inoculation. He suggested that CET solution probably entered into host tissue through hydathodes and natural opening such as stomata. He also reported that the different inoculation methods produced the different types of symptoms on detached leaves of the same age. Because there are little informations regarding to plant age and symptom developed by toxin, a primary purpose of this experiment is to determine the effects of different plant ages on symptom development upon crude extract toxin and purified toxin inoculated on detached leaves and on rice seedlings.

MATERIALS AND METHODS

Preparation of crude extract toxin (CET)

The modified Fries' medium was used to cultivate *P. oryzae* for toxin production. The composition of medium was as follows: sucrose 30g, ammonium tartrate 5 g, ammonium nitrate (NH_4NO_3) 1g, potassium dihydrogen phosphate (KH_2PO_4) 1 g, magnesium sulphate ($MgSO_4.7H_2O$) 0.5g, sodium chloride (NaCl) 0.1 g, calcium chloride ($CaCl_2$) 0.1 g, yeast extract 0.5 g and distilled water is 1L. After the chemicals were thoroughly mixed and dissolved in distilled water, 150 ml of medium were placed into each of 250 ml flask containing 1 g rice seeds. The flasks were capped with cotton plugs and sterilized by autoclaving.

The sterilized modified Fries' medium was then inoculated by mycelium of Khon Kaen isolate of *P. oryzae*. The inoculated medium was incubated at 25°C on an electric mechanical shaker at 200 rpm for 2 weeks. After incubation, the medium was filtered through three layers of cheese cloth to obtain culture filtrate. The volume of this filtrate was reduced to about one tenth of the original volume by using a rotary evaporator. The concentrated culture filtrate was then deproteinized by adding equal volume (1:1) of methanol and kept overnight at 5°C. The sediment was filtered through Whatman No.1 filter paper. The methanol and the water portions were evaporated under rotary evaporator. The viscous brown colour crude extract toxin was obtained and preserved in a refrigerator for further use.

Toxin purification : Concentrated culture filtrate of *P. oryzae* was purified by following the procedure described by Singburaudom *et al.* (1995). It was adjusted to pH 4.5 and then extracted with 50 ml of diethyl ether many times. Two fractions, water phase and ether phase, were obtained. Water phase was discarded because it was not composed of toxic substances. (Singburaudom *et al.*, 1995). Ether phase was evaporated to remove solvent and methanol was added at a ratio 1:1 by volume to deproteinize and the sediments were discarded by filtration. After filtration, solvent was then removed by evaporating and viscous substance and crude extract were obtained. Crude extract was diluted with ethyl acetate and subjected to thin layer chromatography with 100 g of silicic acid followed by step wise elution with a n-hexane-ethyl acetate-acetic acid system. (Umetsu *et al.* 1972). By following this procedure, viscous oily substance and instable substance were obtained. Only instable substance was composed of tenuazonic acid being used for furthur purification. It was combined with $FeCl_3$ to obtain more stable tenuazonic acid in the form of copper complex with green color. Physical structure of purified tenuazonic acid was identified by comparing with standard tenuazonic acid of *Alternaria alternata* under UV light.

Inoculation procedures: The detached leaf technique was employed for toxin inoculation. The second or third leaves from the top of rice plant were detached. Tips and the lower portions of leaf were cut off to retain a 7-8 cm leaf segment from each leaf. The leaf segments were surface sterilized with 10% clorox for 1 to 2 minutes and washed three times in sterile water. Then, each leaf segment was placed on a glass slide with ends fixed by cellulose tape and placed in a moist petridish. In the middle of each leaf a wound was made with a needle and 30 μl of toxin were dropped over the wounded area by a micropipette. The inoculated leaves were incubated for 5 days at 25-28°C under light for symptom development.

Assessment of toxin reaction : The response of rice leaves to toxin was assessed by measuring lesion width, lesion length, and length of running lesion. Typical symptoms produced by toxin were described by following the criteria of blast symptom and it was considered to be the same for assessing the sensitivity to toxin.

Effects of plant age on symptom development upon toxin inoculation

Plant materials: Four rice varieties, HY-71, DP, NMS-4, and KDML 105 classified as resistant, moderately resistant, susceptible and highly susceptible, respectively were planted in clay pots at an interval of 10 days to obtain 20, 30, 40, 50 and 60 day old plants.

Toxin inoculation: The CET was diluted in distilled water to 25% concentration (w/v) and tenuazonic acid was diluted in distilled water to the concentration of 1000 ppm which was considered to be appropriate for toxin inoculation (Chaudhary *et al*., 1995, Singburaudom *et al*., 1995). The experiment was carried out by a factorial in RCB design with four replications, one leaf sample in each replication. The sensitivity of leaves to toxin was recorded by the procedure mentioned above.

Effects of crude extract toxin concentrations on symptom development on rice seedlings

The experiment was carried out in a split-split plot design with five replications to determine the effects of CET concentrations for symptom development on rice varieties at different plant ages. Fourteen and twenty-one day old seedlings of rice varieties HY-71 and KTH-17 were inoculated by spraying CET at the concentrations of 5, 10, 15, 20 and 25% (w/v). The CET concentrations were prepared by serial dilutions in distilled water and distilled water was used for control. CET solutions were sprayed uniformly over the seedling by a small hand plastic sprayer making very fine spray drifts. Then the inoculated plants were coverd by plastic sheets to protect them from rain and wind. Symptoms were observed 5 days after inoculation by estimating % severity of necrotic spot produced on rice leaves.

RESULTS

Effects of plant age on symptom development upon crude extract toxin inoculation

The symptoms produced by inoculation of 25% CET of *P. oryzae* on four rice varieties at five plant ages are shown in Figure 1. Sizes of lesion were also recorded by measuring lesion width, length and running lesion length. The results of the study are as follows:

a. Effect of plant age on lesion width: The analysis of variance of lesion width produced on rice leaves at different plant ages showed a highly significant difference. The result indicated the increase in lesion width was in accordance with plant ages. The largest mean lesion width of 0.36 cm was produced on 60 day old plants whereas the smallest mean lesion width of 0.25 cm was produced on those from 30 day old plants (Table 1). Rice variety DP produced the largest mean lesion width of 0.34 cm while the smallest width of 0.25 cm was found on the variety KDML 105. However, the analysis of variance in regarding to lesion width among variety showed non significant difference. A highly significant interaction effect between plant age and variety was found for lesion width. Variety DP at plant age 50 days produced the largest lesion width of 0.47 cm. It indicated that different genotypes gave different reactions to CET

Table 1 Mean lesion width in cm produced on four rice varieties at five plant ages by crude extract toxin from *P.oryzae* .

Variety	Plant age (DAS)					Mean
	20	30	40	50	60	
HY-71	0.22[1/]	0.14	0.30	0.36	0.45	0.29
DP	0.42	0.19	0.30	0.47	0.32	0.34
NMS-4	0.20	0.34	0.25	0.40	0.37	0.31
KDML 105	0.20	0.32	0.24	0.19	0.32	0.25
Mean	0.26b[2/]	0.25b	0.27ab	0.35a	0.36a	
CV (%)	33.82					
F-test						
Plant age	*					
Variety	ns					
Interaction	**					

[1/] Any two means having the same letter are not significantly different at the 1% level of probability
[2/] Any two means having the same letter are not significantly different at the 5% level of probability
DAS = Days after seeding, ns = non significant different, * = Significantly different at 5% level of probability, ** = Significantly different at 1% level of probability.

when applied at different plant ages. On rice variety KDML 105, there was no difference in lesion width among five plant ages compared with the reaction on other varieties, where significantly different lesion width among plant ages was found.

b. Effect of plant age on lesion length: Table 2 presents the data on the effect of rice plant age on lesion length after inoculating by CET. The analysis of variance of lesion length produced on different plant ages showed significant difference. The result indicated that the lesion length increased with increase of plant age. The smallest mean lesion length of 0.48 cm was produced on leaves from 30 day old plants. On rice variety DP produced the largest mean lesion length of 0.59 cm where as the smallest of 0.49 cm was on the variety HY-71. The analysis of variance for lesion length among varieties showed a non significant difference. However, highly significant interaction between plant age and variety was found. The largest lesion length of 0.81 cm was on rice variety KDML 105 at the age of 60 days old.

c. Effect of plant age on running lesion length:

Table 3 represents the results on the effect of rice plant ages on running lesion length. The analysis of variance of running lesion length produced by CET on rice of different plant ages showed highly significant difference. The largest mean running lesion length of 1.15 cm was produced on leaves from 20 day old plants while the smallest one of 0.79 cm was produced on those from 30 day old plants. The analysis of variance of running lesion length among varieties showed non significant difference. However, the largest mean running lesion length of 1.12 cm was produced on the rice

Table 2 Mean lesion length in cm produced on four rice varieties at five plant ages by crude extract toxin from *P.oryzae*.

Variety	Plant age (DAS)					Mean
	20	30	40	50	60	
HY-71	0.37[1/]	0.40	0.46	0.56	0.64	0.49
DP	0.72	0.37	0.64	0.67	0.55	0.59
NMS-4	0.47	0.55	0.50	0.65	0.57	0.55
KDML 105	0.50	0.62	0.50	0.45	0.81	0.58
Mean	0.51b[2/]	0.48b	0.52ab	0.58ab	0.64a	
CV (%)	24.39					
F-test						
Plant age	*					
Variety	ns					
Interaction	**					

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DAS = Days after seeding, ns = non significantly different, * = Significantly different at 5% level of probability, ** = Significantly different at 1% level of probability.

variety HY-71 where as the smallest one of 0.9 cm was on variety DP. A highly significant effect between plant age and variety was found. The largest running lesion length of 1.62 cm was produced on leaves from variety HY-71 at 60 days old.

Effects of plant age on symptom development upon tenuazonic acid inoculation:

a) Effect of plant age on lesion width : The lesion width produced by tenuazonic acid when applied on rice leaves of four varieties at different plant age are presented in Table 4. The analysis of variance indicated non significant difference of lesion width among 5 different plant ages, four varieties and among interactions between rice variety and plant age. The results suggested that different genotypes gave the same reaction to tenuazonic acid when applied on rice leaves at different plant ages. However, variety HY-71 which classified as resistant, produced the smallest lesion width of 0.16 cm compared with NMS-4 and KDML 105, which classified as susceptible, produced larger lesion width of 0.18 cm and 0.19 cm, respectively. Non significant difference among the lesion width produced on rice leaves at different plant ages indicated no of plant ages influencing on symptom development upon tenuazonic acid inoculation.

b) Effect of plant age on lesion length : Data on the effect of tenuazonic acid on lesion length of four rice varieties at 5 different plant ages indicated the same result as the effect on lesion width (Table 5). The analysis of variance suggested non significant difference of lesion length among 5 different plant ages. The different plant ages gave the same reactions to tenuazonic acid when applied

Table 3 Mean running lesion length in cm produced on four rice varieties at five plant ages by crude extract toxin from *P.oryzae*.

Variety	Plant age (DAS)					Mean
	20	30	40	50	60	
HY-71	1.07[1/]	0.57	1.07	1.27	1.62	1.12
DP	1.10	0.50	1.02	0.82	1.07	0.90
NMS-4	1.10	1.10	0.92	1.12	0.92	1.03
KDML 105	1.35	1.00	1.00	0.77	0.90	1.00
Mean	1.15a[2/]	0.79c	1.00ab	0.99b	1.12a	
CV (%)	28.17					
F-test						
Plant age	*					
Variety	ns					
Interaction	**					

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Table 4 Mean lesion width in cm produced on four rice varieties at five plant ages by tenuazonic acid from *P.oryzae*.

Variety	Plant age (DAS)					Mean
	20	30	40	50	60	
HY-71	0.15	0.14	0.15	0.20	0.17	0.16
DP	0.17	0.14	0.20	0.20	0.19	0.18
NMS-4	0.20	0.20	0.23	0.16	0.13	0.18
KDML 105	0.20	0.11	0.23	0.19	0.21	0.19
Mean	0.18	0.15	0.20	0.19	0.17	
CV (%)	39.66					
F-test						
Plant age	ns					
Variety	ns					
Interaction	ns					

DAS = Days after seeding, ns = non significantly different.

on rice leaves. The largest lesion length of 0.53 cm was produced on 40 day old plants while the smallest lesion length of 0.39 cm was produced on 60 day old plants. The lesion length produced on rice leaves were not significantly different among four rice varieties, although the disease reactions were classified differently among them. Different genotypes at different plant ages gave the same reactions to tenuazonic acid which revealed non interaction effect on symptom development upon tenuazonic acid inoculation.

c) Effect of plant age on running lesion length :

Table 6 represents the data on the effect of rice plant ages on running lesion length after inoculation by tenuazonic acid. The analysis of variance of running lesion produced on different plant ages showed non significant difference. The result indicated that the different plant ages gave the same reactions to tenuazonic acid when applied on rice leaves. However, the longest running lesion length of 1.97 cm was produced on 50 day old plants while the shortest one of 1.20 cm was produced on 60 day old plants. The differences on running lesion length were not found among four rice varieties. However, the longest length of 1.84 cm was produced on susceptible variety NMS-4 and the shortest one of 1.30 cm was produced on variety DP. Significant difference at 5% level of probability of running lesion length was found among different genotypes at different plant ages. On variety NS-4 at the age of 40 days old, running lesion length of 3.33 cm was occured.

Effects of crude extract toxin concentrations on symptom development on rice seedling

The experiment was carried out to determine a suitable concentration of CET for application on rice seedling disease screening. Six concentrations: 0, 5, 10, 15, 20 and 25% of CET were applied on

Table 5 Mean lesion length in cm produced on four rice varieties at five plant ages by tenuazonic acid from *P.oryzae*.

Variety	Plant age (DAS)					Mean
	20	30	40	50	60	
HY-71	0.45	0.36	0.35	0.55	0.42	0.43
DP	0.27	0.67	0.55	0.35	0.40	0.45
NMS-4	0.50	0.35	0.70	0.32	0.27	0.43
KDML 105	0.44	0.22	0.52	0.47	0.45	0.42
Mean	0.42	0.40	0.53	0.43	0.39	
CV (%)	52.34					
F-test						
Plant age	ns					
Variety	ns					
Interaction	ns					

DAS = Days after seeding, ns = non significantly different.

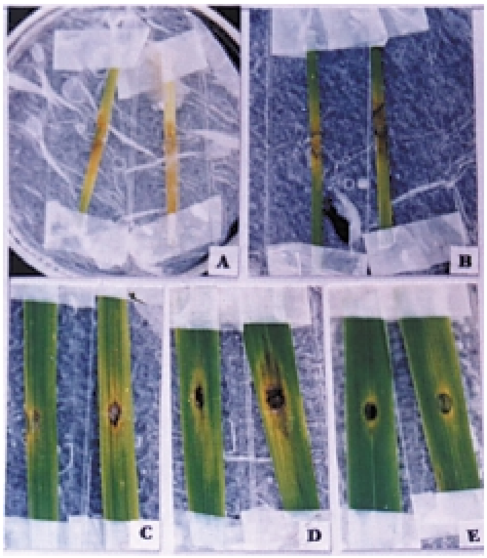

Figure 1 Symptoms produced by crude extract toxin on rice leaves at different plant ages. A, B C, D and E are symptoms on 20, 30, 40, 50 and 60 day old plants, respectively.

two rice varieties at two plant ages of 14 and 21 days old. Observation on disease severity was recorded from 2nd and 3rd leaf of the plant. The result of the experiment is represented in Table 7 and Figure 3. The result indicated that the highest mean disease severity of 47.07% was produced by 25% concentration of CET across the plant ages. Remarkable decline in disease severity was recorded with decrease in CET concentrations. Analysis of variance showed a highly significant difference on disease severity among six concentrations. The highest disease severity of 18.5% was produced on 21 day old plants and analysis of variance showed highly significant difference between two plant ages. Interaction effect between plant age and concentration was found to be significantly different. The highest disease severity of 50.3% was produced by 25% concentration on 21 day old plants, and disease severity was decreased by decreasing of CET concentration in both plant ages. Two rice varieties in this test showed non

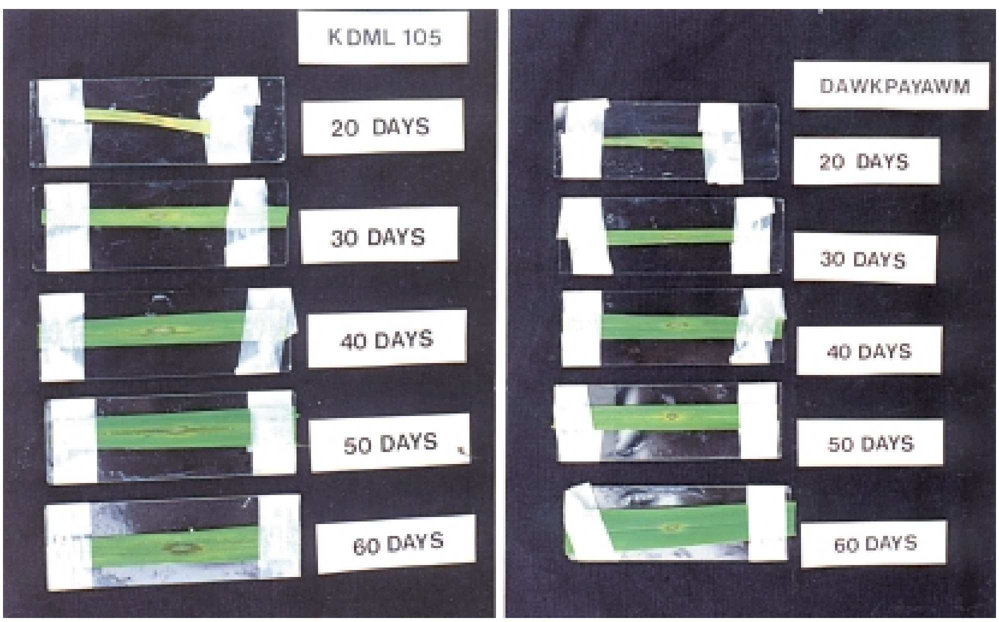

Figure 2 Symptoms produced by tenuazonic acid on diferent plant ages of two rice varieties.
A: Symptoms produced on KDML 105 susceptible rice variety.
B: Symptoms produced on DP resistant rice variety.

Table 6 Mean running lesion length in cm produced on four rice varieties at five plant ages by tenuazonic acid from *P.oryzae.*

Variety	Plant age (DAS)					Mean
	20	30	40	50	60	
HY-71	1.33abc[1/]	1.03abc	1.13abc	2.38abc	1.73abc	1.52
DP	1.43abc	1.93abc	0.78bc	1.25abc	1.10abc	1.30
NMS-4	1.53abc	1.28abc	3.33a	1.40abc	0.65bc	1.84
KDML 105	1.48abc	0.35c	2.20abc	2.85ab	1.33abc	1.64
Mean	1.69	1.14	1.86	1.97	1.20	
CV (%)	67.73					
F-test						
Variety	ns					
Plant age	ns					
Interaction	*					

[1/] Any two means having the same letter are not significantly different at 5% level of probability
DAS = Days after seeding, * = Significantly different at 5% level of probability, ns = non significantly different

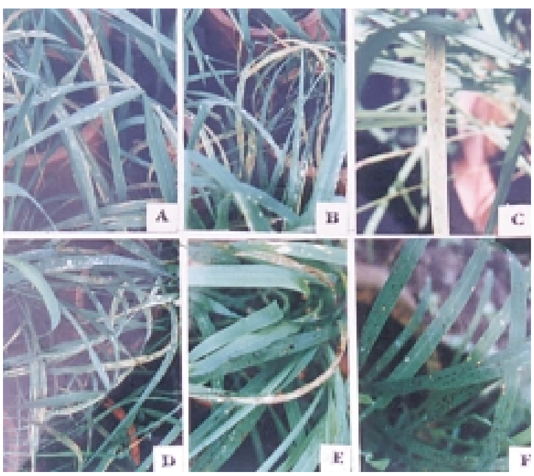

Figure 3 Symptoms produced by CET concentrations on two and three week old seedlings of rice. A, B, and C, are symptoms produced by 25, 20, 15, concentration on 2 week old seedlings. and D, E, and F are on 3 week old seedlings.

significant differences on reaction to CET when it was applied on rice seedlings.

DISCUSSION

Effects of plant age on symptom development by CET inoculation investigated on detached leaves and those from 20-30 day old plants were found more sensitive to CET, manifested by yellowing of leaves, possibly due to absorption and diffusion of toxin solution through the leaf surface (Figure 1). This indicated the suitability of 20-30 day old plants to be used for inoculation of the whole plants. Smaller size of lesions on leaves of younger plants could be due to their small leaf size. Although newly emerged young leaves were detached from plants of all ages. However, it was observed that as the plant age increased, the size of lesion width, length and running lesion length also increased (Figure 1).

The reason might be that CET was retained as drops and did not diffuse over whole leaf area. Despite the selection of newly emerged leaves of all plant ages, the size of leaves from different ages varied considerably. Roumen (1992,1994) found that in case of pathogen infection, the average size of the lesion sporulating area also varied with leaf ages and subsequent decrease in the size of lesion sporulating area with increase of leaf age depended strongly on the genotype. This phenomenon might have impact in the case of toxin inoculation. The result suggested that leaves from 40 day old plants would be suitable for detached leaf inoculation.

Effects of plant age on symptom development upon purified tenuazonic acid inoculation was also investigated on detached leaves. It was found that the size of leaves varied considerably with different plant ages. Symptoms produced on leaves of 20 day old seedlings were manifested more yellowing than those of the older ones. However, the lesions sizes on leaf at all plant ages were not found significantly different compared with the significant difference among lesion size of all plant ages after CET inoculation. In general, size of lesions produced by CET were larger than those produced by tenuazonic acid

Table 7 Mean disease severity (%) produced by six concentrations of CET on rice seedling at two plant ages.

Concentration	Plant ages (DAS)		Mean
	14	21	
0	0.00d[1/]	0.00d[1/]	0.00d[1/]
5	0.45d	0.30d	0.42d
10	0.85d	1.15d	1.00d
15	19.25c	22.95c	18.62c
20	26.90b	39.00b	32.95b
25	43.85a	50.30a	47.07a
Mean	15.00b[2/]	18.50a	16.75
CV (%)variety	23.11		
CV (%)Age	24.62		
CV (%)Concentration	24.44		
F-test			
Variety	ns		
Age	**		
Concentration	**		
Variety x Age	ns		
Variety x Concentration	ns		
Age x Concentration	**		

[1/] With in columns, means with the same letter are not significantly different at 1% level of probability.
[2/] With in columns, means with the same letter are not significantly different at 1% level of probability.

Table 8 Comparison of lesion width, length and running lesion produced on detached leaf rices after inoculating by CET and purified tenuazonic acid toxin.

Plant ages	Width (cm)		Length (cm)		Running lesion length (cm)	
	CET	Tenu [1/]	CET	Tenu	CET	Tenu
20	0.26b[2/]	0.18	0.51b	0.42	1.15a	1.69
30	0.25b	0.15	0.48b	0.40	0.79c	1.14
40	0.27ab	0.20	0.52b	0.53	1.00ab	1.86
50	0.35a	0.19	0.58ab	0.43	0.99b	1.97
60	0.36a	0.17	0.64a	0.39	1.12a	1.20
F-test	*	ns	*	ns	*	ns

[1/] = tenuazonic acid
[2/] = Means within columns with the same letter are not significantly different at 5% level of probability.

(Table8). It was probably due to the differences in toxin concentrations. In case of CET at concentration of 25% (w/v), it might be composed of higher amount of toxin substances than tenuazonic acid at 1000 ppm concentration. However, the symptom produced by tenuazonic acid was very close to typical blast symptom and susceptible varieties, KDML105 and NMS-4, showed more sensitive to tenuazonic acid than resistant varieties, DP and HY-71. The differences in degree of sensitivity manifested by lesion size and degree of yellowing on youngest leaves.

The investigation on effect of CET concentrations on symptom development on whole plants was carried out to determine the appropriate concentration of CET and age of rice seedlings to apply in screening programs for blast resistance. The result on disease severity by spray inoculation of CET showed higher disease severity on three week old seedlings compared with two week old seedlings. However, it was observed that two week old seedlings were more sensitive to CET, since it caused yellowing, wilting and drying up of leaves. The disease severity was recorded on the basis of necrotic spots produced on leaves. Three week old plants had larger leaves than two week old plants and the necrotic spots were more visible than the later. Hence, more necrotic area was recorded on three week old leaves, while those two week old plants showed severe yellowing and wilting which caused difficulty to estimate necrotic spot. Data on symptom severity produced by various concentrations of CET indicated that 25% could be suitable for inoculation on 2-3 week old seedlings. This was supported by the significant interaction effect between plant age and concentration.

LITERATURE CITED

Chaudhary, R.N., N. Singburaudom, T. Sommartya, and E. Sarobol. 1995. Pathogenicity determination of crude extract toxin produced by the fungus *Pyricularia oryzae* Cav. the causal agent of rice blast. Kasetsart J. (Nat. Sci). 29:498-507.

Iwasaki, S., H. Muro, S. Nozoe, and S. Okuda.

1972. Isolation of 3,4-dihydro-3,4,8-trihydroxy-1(2H)-napthalenone and tenuazonic acid from *Pyricularia oryzae* Cav. Tetrahedron Letter No. 1:13-16.

Kozaka, T., M. Tsuchizawa, M. Hanauf and M. Watanabe. 1985. Phytotoxic glycopeptide inducing white head of rice plant produced by *Pyricularia oryzae*. Annuals of the Phytopathological Society of Japan 51:199-205.

Roumen, E.C. 1992. Effect of leaf age on components of partial resistance in rice to leaf blast. Eyphytica 63:271-279.

Roumen, E.C. 1994. A strategy for accumulating genes for partial resistance to blast disease in rice within a conventional breeding program, pp. 245-265. *In* R.S. Zeigler, S.A. Leong and P.S. Teng (eds.). Rice blast disease. CAB International and IRRI.

Singburaudom, N., Y. Ratapha, P. Chantharathin and V. Rakvidhyasastra. 1995. Pathotoxin tenuazonic acid and its role on rice blast disease. Paper presented at the International Conference on Biotechnology Research and Applications for Sustainable Development (BRASD) on August 7-10,1995 Central Plaza Hotel, Bangkok, Thailand. 6p.

Tamari,K. and J. Kaji. 1959. The accumulation of coumarin in stunted rice plant caused by the ill-effects of piricularin. J. of the Agr. Chem. Soci. of Japan 33:181- 183(Ja).

Umetsu, N., J. Kaji, and K. Tamari. 1972. Investigation on the toxin production by several blast fungus strains and isolation of tenuazonic acid as novel toxin. Agr. Biol. Chem.36 :859-866.

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