TEMPERATURE RESPONSE OF *PIRICULARIA ORYZAE* CAV. ISOLATED IN DIFFERENT SEASONS IN TAIWAN[1]

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Introduction

Plant pathogens in the tropics and subtropics perpetuate more generations in the same span of geologic time than those in the temperate zone. Consequently greater chances for the former may be expected to develop new mutants and different races. The effect of environment, particularly temperature-moisture combination and temperature alone, on distribution or limitation of many plant diseases in these area is well documented (Wellman, 1962). Hashioka (1950) studied the differences of the mode of prevalence of the rice blast between the temperate and tropical regions. The results led him to conclude that the prevalence in the temperate region was entirely controlled by the growth of the fungus, whereas in the tropical region the prevalence was largely controlled by the resistance of host. From the results obtained in the studies of varietal reactions of rice to blast, Ou (1963) suggested that the races of the fungus occurring in the different countries were different since many *japonica* varieties, susceptible in Japan and Taiwan, were found to be resistant in Thailand and Philippines. Nevertheless, many *indica* varieties which were considered to be an important source of resistance for breeding in Japan and Taiwan were shown to be susceptible in the tropics.

In Taiwan, studies on the physiologic races of the blast fungus, *Piricularia oryzae* Cav., (Hung and Chien, 1961; Hung *et al.*, 1961; Chien *et al.*, 1963; Kou *et al.*, 1963) were stimulated by the field observation made by Li (1957). He reported that 5 out of 20 rice varieties tested in the uniform blast nurseries of different locations revealed a striking difference in the blast reactions. It is possible that the temperature and seasonal effects on the prevalence of leaf blast are related to the growth of the fungus in this area inasmuch as 34

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monospore isolates of *P. oryzae* responded differently to the suboptimal temperatures tested. This was substantiated by the studies reported here.

Materials and Methods

Thirty four isolates of *P. oryzae* used were obtained from the lesions of the diseased leaves collected from several parts of this island (Kou *et al.*, 1963). Among the isolates, 11 monospore isolates were collected in the first crop and 23 isolates in the second crop seasons. All the cultures were maintained on potato dextrose agar (PDA) at 5°C (Riker and Riker, 1936). The inocula were made by cutting the margin of the colonies which were previously incubated on PDA at 28°C for a week. Inoculated agar plates and containers with liquid medium were incubated at a given temperature to take the data. Growth rate of the fungus was taken every two days for 10 days following inoculation in Petri plate containing 20 ml of medium with 2% agar. Amount of growth was based on the oven-dry weight of the washed mycelial mass in each container when liquid medium was applied as the surface culture. Triplicate was made for each isolate tested.

Results

Effect of temperature on the linear growth

The growth rate of the 34 isolates on potato-dextrose agar and potato-sucrose agar indicated that the optimum temperature for the linear growth was 28°C for all the isolates obtained from both crops. At 34°C, however, the growth rate of the second crop isolates was superior to that of the first crop isolates, although the isolates showing intermediate response were also found at this temperature. Five isolates showing significant response are shown in Fig. 1.

In order to clarify the results more definitely, the statistical methods were employed. The results (Table 1) indicate that effects of temperature and medium are significant at 1% level, and the interaction between AC (temperature × medium), BC (isolates × medium), ABC (temperature × isolates × medium) are also recognized at the same level, while the interaction of AB (temperature × isolates) is significant at 5% level.

To study the response of the isolates to the temperature and the media, t value was computed from different temperatures on different media. The results (Table 2) shows that the different growth rates of isolates obtained from 1st and 2nd crops are highly significant at 34°C regardless of different culture media used. Differences of the growth rates between the isolates obtained from two crop-seasons were secured by using multiple range test.

Table 3 shows that response of the isolates from both crop-seasons are the same at the different temperatures tested, i. e. 28°, 31°, 34°, and 37°C. Comparison of these temperatures indicated that the temperature of 34°C was more suitable to distinguish between the isolates obtained from 1st and 2nd crops than any other temperatures examined.

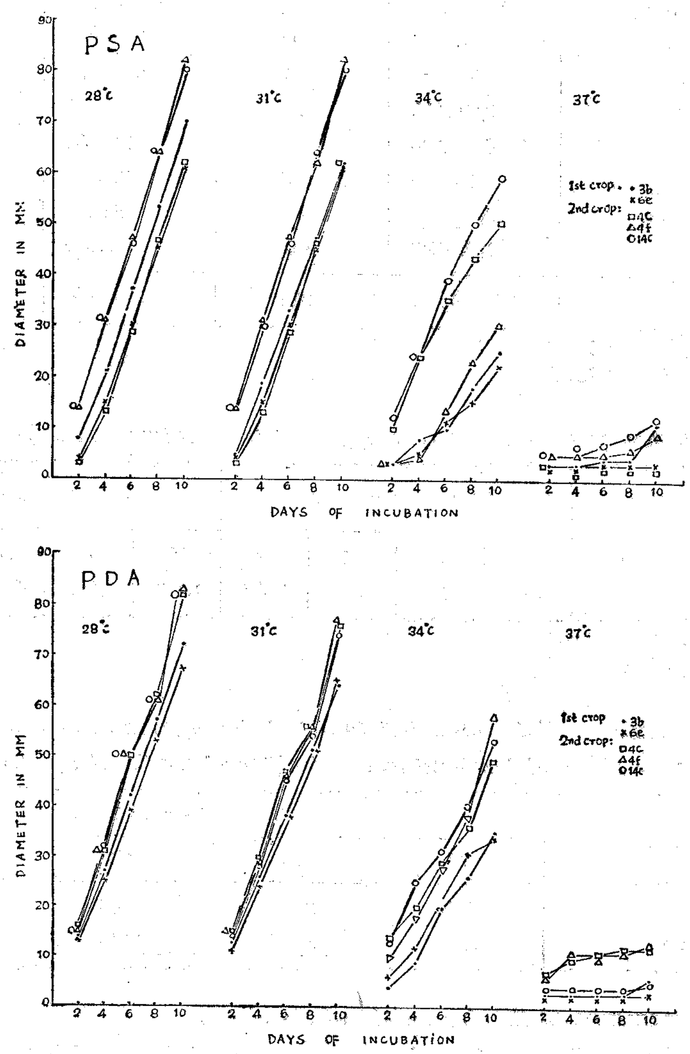

Fig. 1. Showing the effect of temperature on the growth of the isolates of *Piricularia oryzae* collected in the 1st and 2nd crop seasons growing on potato sucrose agar (PSA) and potato dextrose agar (PDA).

Table 1. *Analysis of Variance for 3 factors factorial in a randomized complete block design.*

Source of Variation	Sum of squares	Degrees of freedom	Mean squares	F
Replications	1.120	2	0.560	0.270
Treatments	343,461.020	271	1,267.383	2.366
A (Temperature)	302,165.187	3	100,721.729	414.763**
B (Isolates)	17,249.187	33	522.702	2.124
C (Medium)	4,095.079	1	4,095.076	11.803**
Interaction AB	8,032.979	99	81.141	1.551*
Interaction BC	6,255.254	33	189.553	36.242**
Interaction AC	485.479	3	161.826	30.941**
Interaction ABC	5,177.855	99	52.301	25.273**
Experiment error	1,535.547	742	2.069	
Total	344,997.687	815	4,233.104	

* 5% significant level
** 1% significant level

Table 2. *Values of t computed from different temperatures on different media.*

Medium	Potato dextrose agar				Potato sucrose agar			
Temperature (°C)	28	31	34	37	28	31	34	37
t value	2.500*	1.196	2.888**	1.392	1.129	1.147	4.197**	2.005

* 5% significant level
** 1% significant level

Table 3. *Using multiple range test to detect the differences of growth rates betweeu different temperatures and different media at 5% level.*

Medium	Temperature (°C)			
	37	31	28	34
PDA	1.971	3.451	5.305	7.054
PSA	0.960	3.042	3.735	9.809

Effect of temperature on the dry weight of mycelium.

The surface cultures of 10 isolates were carried out with potato sucrose solution and potato dextrose solution. Growth of the fungus was based on measuring the oven-dry weight of mycelial mass in a 125 ml Erlenmeyer flask with 20 ml culture solution. Six days old cultures were harvested by pouring mycelial mass out of flask into a funnel with a filter paper, then they were throughly washed with distilled water and finally dried in a air oven at 100°C for 20 hours. The dry weight of the mycelial mass was measured until the constant weight was obtained.

Analysis of variance (Table 4) shows that the effects of temperature and isolate, the interaction between AC (temperature×medium), ABC (temperature ×isolate×medium), are recognized at 1% level signification, while the interaction of AB (temperature×isolate) is at 5% level.

Table 4. *Analysis of Variance for 3 factors factorial in a randomized complete block design.*

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Squares	F
Replications	0.0007	$r-1=2$	0.00035	35.0000**
Treatments	1.1176	$t-1=79$	0.00141	
A (Temperature)	1.0784	$a-1=3$	0.35946	1,283.7857**
B (Isolate)	0.0224	$b-1=9$	0.00248	8.8571**
C (Medium)	0.0023	$c-1=1$	0.00232	2.6976
Interaction AB	0.0069	$(a-1)(b-1)=27$	0.00025	1.9230*
Interaction BC	0.0015	$(b-1)(c-1)=9$	0.00016	1.2307
Interaction AC	0.0025	$(a-1)(c-1)=3$	0.00083	6.3846**
Interaction ABC	0.0036	$(a-1)(b-1)(c-1)=27$	0.00013	13.0000**
Experiment error	0.0031	$(r-1)(abc-1)=158$	0.00001	
Total	1.1112	$rabc-1=239$	0.00464	

* 5% significant level
** 1% significant level

The values of *t* computed from different temperatures on different media (Table 5) indicate that difference in amounts of the growth of the isolates obtained from both 1st and 2nd crop seasons is highly significant at any tested temperature (28°, 31°, 34°, and 37°C) regardless of the media used.

Table 5. *Value of t computed from different temperatures on different media.*

Medium	Potato dextrose agar				Potato sucrose agar			
Temperature (°C)	28	31	34	37	28	31	34	37
t value	10.3389**	5.0847**	12.7118**	17.2881**	13.1355**	7.6271**	10.0847**	5.7627**

** 1% significant level

By using multiple range test, the comparision of these temperatures as shown in Table 6 indicate that 37°C is more suitable to distinguish between the isolates of 1st and 2nd crops incubated in PDA medium than any other temperatures tested, while in PSA medium the suitable temperature is found to be at 28°C.

Table 6. *Using multiple range test to detect the differences of grvwth rates between different temperatures and different media at 5% level.*

Medium	Temperature (°C)			
PDA	31	28	34	37
	0.0060	0.0122	0.0160	0.0204
PSA	37	31	34	28
	0.0068	0.0090	0.0119	0.0155

Discussion

In Taiwan, the prevalence of the leaf blast has been observed to be more severe in the first crop than the second crop. Hashioka (1950) reasoned that the warmer weather intensified the host resistance in second crop since the resistance of the host is enhanced in proportion to the increased air and soil temperatures. However, the experimental data obtained from the present studies showed that the isolates of *P. oryzae* collected in the second crop season grew better than those collocted in the first crop season, as they were incubated at higher temperatuae above the optimal range, regardless of the media applied. This indicates the fact that natural selection may help in the establishment of thermoresistant races of the fungus in the warmer temperature when the second crop of rice is grown.

Edginton and Walker (1957) suggested that strains or geographical isolates of *Verticillium sp.* might respond differently to temperature, though seasonal changes in reactions of barley varieties to different races of *Puccinia graminis tritici* were small among the races tested (Patterson *et al.*, 1957). In this connection, it is worthy to note that Line and Garrett (1962) proved that isolates of *P. graminis tritici* cauld be selected to germinate, infect, and develop at low temperature. However, Bromfield (1961) suggested that the effect of temperature was most likely in the host-parasite complex and not on either of the components independently.

Henry and Andersen (1948) showed that the length of the incubation period required for highest sporulation of *P. oryzae* varied with the temperature, the culture vessel, and to some extent, with the isolate. Nevertheless, spores produced at 32°C developed short, knobby, abnormal germ tubes. Recently, Ono (1962) pointed out that environmental conditions under which the conidia of this fungus produced might effect the virulence of the fungus. Although other factors are involved, temperature may well be the most important one determining the geographical distribution and seasonal change of disease occurrence in Taiwan.

Summary

In Taiwan, the prevalence of the leaf blast has been observed to be more severe in the first crop than the second crop. It is possible that the temperature and seasonal effects on the prevalence of leaf blast are related to the growth of the fungus in this area since 34 monospore isolates of *Piricularia oryzae* responded differently to the suboptimal temperatures tested. The isolates obtained in the second crop grew better than those in the first crop when they were incubated at 34°C.

臺灣稻熱病菌對溫度之反應

曾聰徹　袁宸宣　吳龍溪

臺灣稻熱病的流行於第一期作水稻較第二期作猖獗，其原因係溫度和季節影響發病，而與病原菌本身的生長也有密切的關係。

將稻熱病菌三十四菌株分別作溫度試驗結果發現在34°C溫度下培養時，第二期作所分離之菌株其生長率顯然比第一期作高。

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