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Studies on the Breakdown of Resistance in the Blast Resistant Rice Variety, CO 29 in Tamil Nadu

by

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Introduction: CO 29, a high yielding short duration blast resistant rice variety has replaced the existing susceptible varieties in many parts of the Tamil Nadu. But during winter season 1966, CO 29 variety was found to be severely affected by the blast disease resulting in almost complete loss of crop in many parts of Coimbatore district. During 1967 winter season also, the blast incidence was noticed on CO 29 in many other districts in the Tamil Nadu. Such breakdown in resistance may be due to the change in environment as in the case of 'Polygenic resistance' or due to the development of new races of the pathogen as in the case of 'Single gene resistance' (Walker, 1959). Suryanarayanan (1958) reported that even the resistant varieties when subjected to low night temperature (20°C), showed characteristic blast lesions on inoculation with *Piricularia oryzae* Cav. Padmanabhan (1965) also has reported the occurence of races of *P. oryzae* in India. The present investigation was undertaken to elucidate the factors responsible for the breakdown of resistance to blast in CO 29 rice variety.

Materials and Methods: Five isolates of P. *oryzae* two from blast resistant varieties *viz.*, CO 29 and CO 4 and three from blast susceptible varieties *viz.*, CO 13, ADT 10 and ADT 27 were selected for the study. The isolates from CO 4, CO 29 and ADT 10 were brought into pure culture by monosporic isolations from infected paddy leaves collected from Paddy Breeding Station and Central Farm wetlands, Coimbatore. The isolate from ADT 27 was isolated from infected paddy leaves collected from Thanjavur. The isolate from CO 13 (isolated during 1965) was obtained from the stock culture maintained in the Mycology section, Coimbatore. These cultures were maintained on oats agar medium. The inoculum was multiplied in steamed leaf bits of *Brachiaria mutica* fortified with Richard's solution and used for inoculation on young seedlings.

Seven varieties of rice comprising four resistant varieties *viz.*, CO 4, CO 25, CO 29 and CO 30 and three susceptible varieties, ADT 10, ADT 27

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and CO 13 were raised in pots. The seedlings were grown in four different chambers maintaining temperatures of 17°, 20°, 25° and 30°C. The seedlings were subjected to the different temperatures only during night time and they were exposed to sunlight (30° – 33°C) during day time. Fifteen days old seedlings were inoculated by spraying a heavy spore suspension of the different isolates of the pathogen. The inoculated plants were under observations for disease symptoms and final recording was done on the seventh day. The reactions of the rice varieties to the various isolates of the pathogen were recorded as per the scales suggested in the symposium on the Rice Blast Disease in July 1963 held at Manila. Five scales were used *viz*., highly resistant (—), resistant (*), moderately resistant (**), moderately susceptible (***) and susceptible (****) reactions.

The morphological and cultural characters of all the five isolates of the pathogen were studied. The growth rate and the intensity of sporulation were assessed by growing them in oats agar medium. The spore concentration was assessed using a haemocytometer. The spore measurements were done after mounting them in lactophenol.

The toxin production by the different isolates *in vitro* was assayed by growing them in Czapek-Dox medium. After 15 days incubation the culture filtrates were obtained and used as toxin samples. The toxin production was tested by plumule and radicle inhibition bioassays. Seeds of CO 13 rice variety was used in the bioassay studies. For the plumule inhibition bioassay, germinated seeds with plumule measuring 5 mm. length were selected and spread over sterile filter paper placed in sterilized petri dishes. The filter paper was wetted with 5 ml. of the culture filtrate. Un-inoculated medium and sterile water series were also maintained to serve as control. For the radicle inhibition bioassay, seeds with radicle measuring 5 mm. length were used. After 72 hours, the length of plumule or radicle was measured.

To assess the comparative virulence of the different isolates, eight International differential host varieties of rice obtained from the International Rice Research Institute, Philippines were used. The following eight International differentials were used: Raminad Str. 3, Zenith, Usen, Dular, Kanto 51, Sha-tiao-tsao, Caloro and NP-125. Fifteen days old seedlings were inoculated. The seedlings were exposed to 20°C night temperature alternating with exposure to sunlight during day time (30° – 30°C) for three days before and after inoculation.

Results: The reaction of seven rice varieties to the various isolates of *P. oryzae* at different temperatures were studied and the results are presented in Table 1.

TABLE 1. *Reactions of rice varieties to different isolates of Piricularia oryzae at various temperatures*

Isolate from	Night temperature in °C	Intensity of infection in resistant varieties				Intensity of infection in susceptible varieties		
		CO 4	CO 29	CO 30	CO 25	ADT 27	ADT 10	CO 13
CO 4	17	**	***	***	***	***	****	****
	20	**	***	***	***	***	****	****
	25	—	*	*	*	*	**	**
	30	—	—	—	—	—	—	—
CO 29	17	**	***	***	***	***	****	****
	20	**	***	***	***	***	****	****
	25	—	*	*	*	**	**	**
	30	—	—	—	—	—	—	—
CO 13	17	—	—	—	—	****	****	****
	20	—	—	—	—	****	****	****
	25	—	—	—	—	0	0	0
	30	—	—	—	—	—	—	—
ADT. 10	17	—	—	—	—	****	****	****
	20	—	—	—	—	****	****	****
	25	—	—	—	—	*	*	*
	30	—	—	—	—	—	—	—
ADT. 27	17	—	—	—	—	****	****	****
	20	—	—	—	—	****	****	****
	25	—	—	—	—	*	*	*
	30	—	—	—	—	—	—	—

The isolates from resistant varieties *viz.*, CO 4 and CO 29 produced typical blast lesions in both resistant and susceptibe varieties at 17° and 20°C. At 25°C, only resistant type of reaction was observed in the resistant varieties while the susceptible varieties showed susceptible type of reaction (scale 3) producing small, roundish, necrotic, grey spots. At 30°C, no symptoms were observed on any of the varieties tested. Isolates from the susceptible varieties *viz.*, CO 13, ADT 10 and ADT 27 did not produce any lesions on the resistant varieties, CO 4, CO 25, CO 29 and CO 30 at any of the temperatures tested. But susceptible varieties CO 13, ADT 10 and ADT 27 showed susceptible type of reaction to these isolates at 17° and 20°C. At 25°C, they showed only few specks and at 30°C no sign of any type of infection was observed.

The morphological and cultural characters of the different isolates of the pathogen were studied and the data are presented in Table 2.

TABLE 2. *Cultural characters, sporulation and conidial measurements of various isolates of P. oryzae on oats agar medium*

Isolate from	Colour of the colony	Rate of growth in mm/ 24 hour	Spore concentration in lakhs/ml	Spore size	
				Range Length×breadth in μ	Mean Length×breadth in μ
CO 4	Olive grey	4.6	4.83	20.0–32.5×7.5–10.0	24.9×8.3
CO 29	White	4.7	4.73	18.8–31.3×7.5–10.0	24.1×8.3
CO 13	Black	3.1	3.65	17.5–27.5×7.5–10.0	22.7×8.7
ADT 10	Greyish white	6.0	3.93	16.5–26.3×7.5–10.0	21.8×8.5
ADT 27	Greyish white	6.0	2.33	18.8–30.0×7.5–10.0	23.4×8.7

The isolates from ADT 10 and ADT 27 were found to grow vigorously while isolates from CO 4 and CO 29 showed moderate growth. The isolate from CO 13 exhibited the slowest growth. Differences were also observed in respect of colony colour. Isolates from ADT 10 and ADT 27 varieties produced greyish white colony, the isolate from CO 4 produced olive grey growth, isolate from CO 29 produced a white colony and the isolate from CO 29 produced a black colony. Abundant sporulation was observed with the isolates from the resistant varieties CO 4 and CO 29 while the sporulation was less with the isolates from susceptible varieties. The size of the conidia of the isolates from resistant varieties also differed from the isolates of susceptible varieties. The conidia from CO 4 and CO 29 isolates were slightly longer than those from CO 13, ADT 10 and ADT 27. Conidial size was the smallest in the case of ADT 10 isolate. The results of toxin production are presented in Table 3.

TABLE 3. *Effect of toxin on radicle and plumule inhibition in differnt isolates of Piricularia oryzae*

Culture filtrate of the isolates from	Radicle		Plumule	
	Length in mm** (C.D.=2.8)	% inhibition over un-inoculated medium	Length in mm** (C.D.=4.7)	% inhibition over un-inoculated medium
CO 4	20.9	47.6	15.0	44.5
CO 29	21.4	46.2	15.8	41.5
ADT 27	24.6	38.2	17.5	35.2
CO 13	29.6	25.6	21.6	20.0
Un-inoculated medium	39.8	—	27.0	—
Distilled water	44.0	—	29.2	—

** Significant at 1% level

The isolates from the resistant varieties CO 4 and CO 29 produced greater amount of toxins and they inhibited both radicle and plumule elongations markedly. The isoates from ADT 27 and CO 13 also produced toxins

as evidenced by the inhibition of radicle and plumule elongations, but the degree of inhibition was much less when compared with those from resistant varieties.

The virulence of the various isolates was assessed on eight International differential rice varieties and the results are presented in Table 4.

TABLE 4. *Reactions of International differential rice varieties to various isolates of Piricularia oryzae*

Piricularia oryzae isolates from	International differential varieties							
	Raminad Str. 3	Zenith	Usen	Dular	Kanto 51	Sha-tsiao-tsao	Caloro	NP-125
CO 4	⊕⊕	—	⊕⊕	⊕⊕⊕⊕	—	—	⊕⊕	⊕
CO 29	⊕⊖	—	⊖⊕	⊕⊕⊕⊕	—	—	⊕⊕	⊕
CO 13	—	—	—	⊕⊕	—	—	—	—
ADT 10	—	—	—	—	—	—	—	—
ADT 27	—	—	—	⊕⊕	—	—	—	—

CO 4 and CO 29 isolates behaved similarly inducing susceptible type of reaction in Dular, moderately resistant reaction in NP-125 and highly resistant reaction in Zenith, Kanto 51 and Sha-tiao-tsao. The isolates from CO 13 and ADT 27 produced browning reaction only in Dular and all other varieties were found to exhibit immune reaction. All the varieties showed highly resistant reaction to ADT 10 isolate.

Discussion : The CO 29 rice variety which was considered to be resistant to blast disease so far, has suddenly succumbed to the disease creating serious concern to the scientists as well as the farming public. The various factors that might have contributed for the recent breakdown of CO 29 variety during winter 1966 may perhaps be due to the abnormal prevalence of very low night temperatures during that season. The present studies revealed that at low night temperature of 17° and 20°C, isolates from resistant varieties were found to produce moderately susceptible type of reaction on resistant varieties and highly susceptible type of reaction on the susceptible varieties. On the other hand, the isolates from the susceptible varieties were not able to infect the resistant varieties but produced typical symptoms on the susceptible varieties. In as much as the isolate from CO 29 was able to produce moderately susceptible type of reaction on CO 29 variety which was hitherto considered to be a resistant one, the isolate may be considered as new one possessing greater virulence than the existing isolates of *P. oryzae*. Differences in virulence between isolates of *P. oryzae* from resistant and susceptible varieties have been reported by Nakanishi and Imamura (1956). Suryanarayanan (1958) reported that a night temperature of 20°C alternating with day temperatures

of 30°–35°C favoured infection in both blast resistant and susceptible paddy varieties. Ramakrishnan (1966) observed that CO 29 variety remained resistant at all the three night temperatures *viz*., 20°, 25° and 30°C tested by him. He, however, stated that at 20°C small brown lesions were observed rarely. The lowest temperature of 17°C included in this investigation has not been tested by him. Padmanabhan *et al* (1967) distinguished 16 races and 3 biotypes out of 19 isolates of *P. oryzae* collected from principal rice growing regions of India. The main criteria used by Misra and Das (1965) to distinguish different isolates into races are their morphological and cultural characters and the difference in their pathogenicity. In the present studies differences in growth rate, colour of the colony, sporulation and size of the spores were observed among the various isolates. Variations in colour of aerial mycelium, sporulation, colour production in medium and other cultural characteristics between isolates of *P. oryzae* have been reported by Tochinai and Shimamura (1932). Konishi (1933) and Aoki (1935) have suggested that cultural characters such as colouration of submerged mycelium, formation of aerial hyphae and amount of sporulation could be employed for distinguishing isolates of *P. oryzae* into physiologic forms. The isolate from CO 29 was found to be different from the existing isolates in respect of all the characters mentioned above.

The results in this investigations have also revealed that isolates from the resistant varieties, CO 4 and CO 29 produced greater amount of toxin *in vitro* than the isolates from the susceptible varieties CO 13 and ADT 10. Differences in toxin production between different isolates of *P. oryzae* have been reported by Shanmugam (1963). He has also reported an almost positive correlation between toxin production and pathogenicity in *P. oryzae*. Luke and Wheeler (1955) reported that pathogenicity and ability to produce toxins in *Helminthosporinm victoriae* were closely correlated. It is, therefore, evident that CO 29 isolate was more virulent as evidenced by its higher toxin production than the existing isolates of ADT 10, CO 13 and ADT 27.

The pathogenicity reaction of the International differential rice varieties to the various isolates also showed considerable differences between them. Isolates from CO 4 and CO 29 were found to be similar in producing the same type of reaction and are distinctively different from the other isolates CO 13, ADT 10 and ADT 27. Latterell *et al* (1960) have reported 15 races of *P. oryzae* on the basis of differential reactions on 10 differential host varieties of paddy from a collection of 200 isolates obtained from different parts of the world. It is, therefore, quite evident that the isolate from CO 29 is a new one.

Thus, the findings obtained in this study, clearly indicated the appearance of a new physiologic race of *P. oryzae* quite different from the existing ones.

Summary : The blast resistant rice variety CO 29, suddenly succumbed to the disease in different parts of Coimbatore district during winter seasons of 1966 and 1967. The pathogen was isolated from CO 29 and compared with the existing isolates from the susceptible varieties. The blast resistant varieties CO 4, CO 25, CO 29 and CO 30 when artificially inoculated with the isolates from resistant varieties CO 4 and CO 29 showed typical blast lesions at low temperatures of 17° and 20°. But when the resistant varieties were inoculated with the isolates from susceptible varieties *viz.*, CO 13, ADT 10 and ADT 27, no infection was noticed even at low temperatures.

Variation was observed between the isolates of *P. oryzae* from resistant and susceptible varieties with regard to morphological and cultural characters such as colour of the colony, growth rate, extent of sporulation and size of the conidia. The isolates from the resistant varieties, CO 4 and CO 29 were found to produce greater quantity of toxin when compared with the isolates from the susceptible varieties CO 13, and ADT 27. The reactions of the International differential rice varieties to the various isolates showed considerable differences between them. Isolates from CO 4 and CO 29 behaved similarly in many respects and distinctly different from the other isolates. It was therefore, concluded that the isolate from CO 29 was different from the existing ones and is considered as a new physiologic race of *Piriculraia oryzae.*

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* Originals not seen

Effect of Hot Water Treatment on the Control of Primary Seed Infection in Rice Caused by *Xanthomonas Oryzae*

by

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Introduction : Bacterial leaf blight caused by *Xanthomonas oryzae* (Uyeda and Ishiyama) was reported from India in 1951 from Khopoli area of Bombay. Sulaiman and Ahamed (1955) reported that this disease has caused consideable loss to paddy crop in several parts of Punjab, Uttar Pradesh, Bihar, Orissa, Madhya Pradesh, Maharasthra and Andhra Pradesh in recent years. In Tamil Nadu this disease appeared in the newly introduced high yielding variety Taichung Native 1 in a serious form. This disease was also noticed in some of the improved rice strains under cultivation in Tamil Nadu *viz.*, ADT 27, CO 25, CO 29, CO 30, CO 32 and BAM 3. Srivastava and Rao (1964) reported that seed-borne infection of *X. oryzae* can be eradicated to the extent of 95-100% by soaking infected paddy seeds for 12 hours at room temperature in aqueous solutions of Agrimycin + wettable ceresan (in concentrations of 0.025+0.05%) followed by hot water treatment at 52°-54°C for 30 minutes. With a view to assess the effiicacy of hot water

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