PHYSIOLOGIC SPECIALISATION IN *PIRICULARIA ORYZAE* CAV.

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Abstract

The existence of physiologic races in *Piricularia oryzae* Cav., the causal fungus of blast disease, has been established in India. Sixteen races and three biotypes were distinguished out of 19 isolates tested. Three of the races correspond to races 8, 16 and 25 of the U.S.A.

Varieties like *BJ.* 1, *Zenith*, *Norin*-22, *Te-tep* and *Tadukan* have proved resistant to all the Indian races of *Piricularia oryzae*.

Introduction

A study has been in progress at the Central Rice Research Institute, Cuttack, since 1961 to determine whether *Piricularia oryzae* Cav., the pathogen causing blast disease of rice, occurs as specialized races in India and if so, to determine the number and distribution of such races in the country. The project is financed by the U.S.D.A. from PL-480 funds. A preliminary note of the findings has been already published earlier (Padmanabhan, 1965).

Materials and Methods

Differentials.—The differential varieties, used in identifying the races of *P. oryzae*, were those employed by Latterell *et al.* (1960) in U.S.A. and Goto and Yamanaka (1961) in Japan along with 12 varieties from the genetic stocks at this Institute listed below. The 12 varieties from Cuttack had shown marked differential reaction in the co-ordinated varietal susceptibility trials in India and in the International blast varietal testing programme, organised during 1956–59 (Richharia *et al.*, 1961).

U.S. differentials.—Zenith, C.I. 8970 (S), C.I. 8970 (P), P.I. 201902, P.I. 180061, Caloro (1961–1), Lacrosse (C.I. 8985), C.I. 5309, Wag Wag and Raminad Str. 3.

Japanese differentials.—Ishikari Shiroke, Homare Nishiki, Norin–1, Norin–20, Norin–22, Kanto–51, Aichi Asahi, Ginga, Chokotou, Tadukan, Usen, Yakeiko, Rei-shi-ko and Te-tep.

Indian differentials.—B.J.–1, AC. 1613, S. 67, SM. 6, AC. 1423, AC. 1443, CR. 906, CR. 907, Intan, Bengawan, Mas and Co. 13.

Collection of samples, isolation and purification.—Samples of diseased leaves, necks and nodes of infected rice plants were collected from the principal rice-growing regions of India, mostly through the courtesy of departmental officials in each state and also by personal collection by the authors.

Isolates of *P. oryzae* were obtained by asceptically transferring to Oat Meal Agar pieces of blast infected leaves and necks after sterilizing them in 0·1% solution of mercuric chloride for a minute and washing them in sterile water; Oat Meal Agar used was prepared with traces of Biotin and Thiamine. Cultures were purified by dilution method and single spore isolates were grown and multiplied on Oat Meal Agar with traces of Thiamine and Biotin at 25° C.

Preparation of inoculum.—Spore-suspensions were prepared from 12–15 days old cultures, having at least 6–8 spores per microscopic field under low power magnification. The entire thallus of weakly sporulating isolates was carefully removed from the agar medium and homogenised with water in Waring blender and used for noculation.

Raising seedlings of differentials.—Seedlings of differentials were raised in wooden boxes with partitions, containing 40 kg. field soil, mixed with compost and 30 gm. of superphosphate. Ammonium sulphate was applied in two split doses of 30 gm. and 15 gm., once at sowing and the second, a week before inoculation. Usually three weeks old seedlings were used for inoculation.

Inoculation chambers.—The inoculation chambers used for artificial infection tests were made of wooden frames $5' \times 3\frac{1}{2}' \times 3\frac{1}{2}'$ with glass doors in front and back and with muslin cloth on the sides and top. The front door with glass was provided with additional cloth curtains.

Artificial infection.—Artificial infection tests were carried out in the night when temperature came down below 26° C.; this was mostly during September to November and January to March. For each isolate a separate chamber was used. The spore-suspension of the appropriate isolate was prepared and sprayed upon the seedling leaves through atomizer. To maintain high relative humidity, the cloth was kept wet overnight with the

help of water running through interconnected plastic tubes with holes one inch apart all through. The water came out as a fine spray and kept the cloth wet throughout the night. The inoculations were repeated twice.

Scoring of infection.—The scoring of infection was carried out according to the system in use at the Central Rice Research Institute, since 1948 for varietal susceptibility trials, described in detail by Padmanabhan and Ganguly (1959). This was based upon the type of spots and the number of spots which developed on the most heavily infected leaf in each plant. Methods of scoring based upon the type of spots have been utilised in U.S.A. (Latterell *et al.*, 1960), and Japan (Goto and Yamanaka, 1961). The scoring and the classification of the types in terms of the scores adopted in studies in India, U.S.A. and Japan are described in Table I. It may be seen that they are essentially similar.

TABLE I

Reaction types in use at the Central Rice Research Institute, Cuttack and their corresponding U.S.A. and Japan's reaction types

Reaction types	Classification of reaction types	Corresponding reaction types	
		U.S.A.	Japan
A. Just reddish flecks ..	R = Resistant	0, 1 and 2	0 and 1
B. Minute reddish spots showing no differentiation into distinct zones			
C. Circular spots about 2–3 mm. in diameter with a central ashy zone and a dark purplish-brown margin	MR = Moderately resistant	3	2
D. Broadly spindle-shaped spots only, slightly longer than broad, 3–5 mm. in diameter	S = Susceptible	4 and 5	3 and 4
E. Large distinct spindle-shaped spots with a central ashy zone and marginal zones 3–5 mm. broad and upto several centimetres in length			

Race designation.—A uniform world-wide system of nomenclature for races has not yet been evolved. The Technical Planning Committee for International Blast Programme at the International Rice Research Institute, Manila, 1963, has recommended that national designations should be retained until a uniform system is evolved in the future. In pursuance of this decision the races identified in India have been given Indian Numbers (I.R. Series).

Tests performed.—The following 19 isolates of *Piricularia oryzae* from different centres in India were tested, each being repeated at least 3 times.

(1) Anakapalle, (2) Araku, (3) Deras, (4) Pattambi, (5) Srinagar, (6) Madras–4, (7) Madras–5, (8) Jeypore (O.R.–10), (9) Kanpur, (10) Coochbehar, (11) Tripura, (12) Cuttack, (13) O.R.–8, (14) Kalimpong, (15) U.P.–13, (16) West Bengal–28, (17) Gujarat–1, (18) Hebbal, & (19) Kerala–13.

RESULTS

During 1962–63, six isolates were tested as a result of which four races were distinguished. Further confirmatory tests with them were made during 1963–64, along with the 13 fresh isolates. The reaction of U.S. differentials with 19 isolates is given in Table II.

It is clear from Table II that with the U.S. differential varieties, 13 races could be distinguished out of 19 isolates tested. Three out of the 13 races correspond to races 8, 16 and 25 of the U.S.A. (Latterell *et al.*, 1965).

The reaction of Indian varieties with 19 isolates is given in Table III. The varieties S. 67 and B.J. 1 behaved as resistant, and their reaction is not included in the table. From the available data, it is evident that the varieties, specially AC. 1613, Bengawan, SM. 6, Mas. CR. 906 or CR. 907, AC. 1423 and Intan have shown clear-cut differential reactions and can usefully be employed for differentiating races in India.

With the addition of 4 Indian differentials, *viz.*, AC. 1613, CR. 906, SM. 6 and Mas or Bengawan to the U.S. differentials five isolates, *viz.*, U.P. 13, W.B. 28, Gujarat 1, Hebbal and K.R. 13 belonging to U.S. race 25 can further be distinguished into 4 races, *viz.*, 13, 14, 15 and 16 as shown in Table IV.

As is shown in Table II, three isolates, *viz.*, Cuttack, OR–8 and Tripura belong to race 8 of the U.S.A. These could further be differentiated as 3 different biotypes on the basis of their reaction on three Japanese varieties, *viz.*, Chokotou and Yakeiko or Ginga as given in Table V.

TABLE II

Reaction of U.S. differential varieties to 19 *isolates of* Piricularia oryzae

Isolates	Zenith	La-crosse	Caloro	C.I. 8970 (P)	C.I. 8970 (S)	C.I. 5309	P.I. 180061	P.I. 201902	Wag Wag	Rami-nad Str. 3	Indian races	Corres-ponding U.S. races
Anakapalle	R	S	S	S	S	S	S	S	R	S	1	..
Araku	R	M	S	S	M	S	S	M	R	R	2	..
Deras	R	S	S	S	S	S	S	S	S	R	3	..
Pattambi	R	M	S	S	S	S	S	R	M	S	4	..
Srinagar	R	S	S	S	S	R	S	R	R	R	5	..
Madras–4	R	M	M	M	S	S	S	S	M	R	6	..
Madras–5	R	S	R	R	S	S	R	R	R	R	7	..
Jeypore (O.R.–10)	R	M	S	M	S	S	R	R	R	R	8	..
Kanpur	R	S	S	S	S	S	S	R	R	S	9	..
Coochbehar	R	M	S	S	S	S	M	R	R	M	10	..
Tripura	R	S	S	S	S	S	S	M	R	R	11	8
Cuttack	R	S	S	S	S	S	S	R	R	M	11	8
O.R.–8	R	S	S	S	S	S	S	R	R	R	11	8
Kalimpong	R	S	S	S	S	R	S	S	R	R	12	16
U.P.–13	R	S	S	S	S	S	S	S	S	S	13	25
W.B.–28	R	S	S	S	S	S	S	S	S	S	13	25
Gujarat–1	R	S	S	S	S	S	S	S	S	S	13	25
Hebbal	R	S	S	S	S	S	S	S	S	S	13	25
K.R.–13	R	S	S	S	S	S	S	S	S	S	13	25

N.B.—While identifying the races both R and M were considered as resistant.

TABLE III

Reaction of Indian differential varieties to 19 *isolates of* P. oryzae

Isolates	AC. 1613	CR. 906	CR. 907	Benga-wan	SM. 6	Mas.	AC. 1423	AC. 1443	Intan.	Co. 13
Anakapalle ..	R	R	R	M	S	S	..	...	S	S
Araku ..	..	..	..	S	R	..	M	M	M	S
Deras ..	M	S	S	S	S	S	..	...	M	S
Pattambi ..	...	..	..	M	..	..	R	M	...	S
Srinagar ..	R	..	R	R	R	..	R	S	R	S
Madras–4 ..	..	..	..	S	M	..	S	M	M	S
Madras–5 ..	..	..	..	..	..	..	R	...	...	S
O.R.–10 ..	..	..	..	M	...	..	R	M	M	S
Kanpur ..	..	..	R	S	..	..	S	S	R	S
Coochbehar ..	..	..	R	M	..	..	R	M	M	S
Tripura ..	..	M	..	M	..	..	M	S	..	S
Cuttack ..	..	..	...	..	..	..	S	..	..	S
O.R.–8 ..	M	R	R	S	S	S	..	..	S	S
Kalimpong ..	R	..	R	M	R	R	R	R	R	S
U.P.–13 ..	M	S	S	S	R	S	..	..	M	S
W.B.–28 ..	R	S	S	S	R	S	..	..	R	S
Gujarat–1 ...	M	S	S	S	S	S	..	..	R	S
Hebbal ..	M	R	R	R	R	R	..	..	R	S
K.R.–13 ..	S	S	S	R	M	M	..	..	M	S

TABLE IV

Reaction of 4 varieties with five isolates belonging to U.S. race 25

Isolates	U.S. race	AC. 1613	CR. 906	SM. 6	Bengawan or Mas	Indian race
U.P.–13 ..	25	M	S	R	S	13
W.B. ..	25	R	S	R	S	13
Gujarat–1 ..	25	R	S	S	S	14
Hebbal ..	25	M	R	M	R	15
K.R.–13 ..	25	S	S	R	R	16

TABLE V

Reaction of 3 Japanese varieties with three isolates belonging to I.R. II (U.S. Race–8)

Isolate	Chokotou	Yakeiko or Ginga		Biotype	
Cuttack ..	S	R	R	1R	11A
O.R.–8 ..	M	S	S	1R	11B
Tripura ..	R	M	R	1R	11C

The reaction with 19 isolates on the Japanese set of differentials is presented in Table VI. It may be seen that at least 12 races could be distinguished amongst the 19 isolates on the basis of the reaction of Japanese differentials.

Distribution of races.—The geographical distribution of the sixteen races so far identified is given below.

Region	Name of the States	Races of *P. oryzae* found
Eastern ..	Tripura	I.R. 11 C.
	West Bengal	I.R. 10, 12 and 13.
	Orissa	I.R. 3, 8, 11 A and 11 B.
Northern ..	Uttar Pradesh	I.R. 9 and 13.
	Jammu and Kashmir	I.R. 5.

Region	Name of the States	Races of *P. oryzae* found
Western ..	Gujarat	I.R. 14.
Southern ..	Andhra Pradesh	I.R. 1 and 2.
	Madras	I.R. 6 and 7.
	Mysore	I.R. 15.
	Kerala	I.R. 4 and 16.

DISCUSSION

The above study has clearly established that *Piricularia oryzae*, the causal organism of blast disease of rice, exists as physiologically specialized races in India. Three of the U.S. races, *viz.*, U.S.–8, 16 and 25 have also been identified in India. It is of interest to record that race 25, which arose as a cultural variant of 16, has been obtained under natural conditions in India.

Though three out of sixteen races are common between U.S.A. and India, only one race I.R.–13 has been identified both in Northern and Eastern region. As the study progresses, races common amongst the different regions of India may be found.

It has been shown by a number of workers that the disease expression in blast of rice, is very much influenced by environmental factors and therefore, it is very essential that the infection tests are carried out under strictly standardised conditions, if the results obtained in different testing centres are to be comparable. For the establishment of identity of races reported from testing centres, this is absolutely necessary.

Of the many factors which would require rigid standardization in such artificial infection tests, the temperature conditions under which the varieties are raised are most important. The effect of temperature on the reaction of the host (Hashioka, 1950; Suryanarayanan, 1958) is so significant that unless this is standardised, the establishment of identity of races differentiated in various testing centres in a country or in different countries may be very difficult.

Similarly, the level of nitrogen under which the seedlings are raised for the tests is another important factor which needs to be standardised also. It may be standardised at 100 lb. N/acre.

TABLE VI

Reaction of Japanese differentials against isolates of Piricularia oryzae

	Isolates	Tetep.	Tadukan	Zenith	Norin No. 1	Norin No.20	Norin No. 22	Homare-Nishiki	Ginga	Ishikari-shiroke	Yakei-ko	Reishi-ko	Choko tou	Aichi Asahi	Kanto —51	Race
1.	Cuttack ..	R	R	R	..	R	R	R	R	R	R	R	S	S	S	1
2.	Madras–4 ..	..	R	R	M	R	...	R	R	M	S	S	S	S	S	2
3.	Araku ..	M	R	R	..	R	R	R	R	R	S	M	S	S	S	3
4.	Tripura ..	R	R	R	M	R	R	R	M	R	R	S	R	S	S	4
5.	Pattambi ..	..	..	..	R	..	R	M	R	R	M	M	S	S	S	S	5
6.	Deras ..	R	R	R	M	R	R	S	M	S	S	S	S	S	S	6
7.	K.R.–13 ..	R	R	R	R	R	R	R	S	S	S	S	R	S	S	7
8.	Hebbal ..	R	R	R	R	R	R	R	S	S	S	S	R	S	S	7
9.	Gujarat–1 ..	R	R	R	R	R	R	M	S	M	S	S	R	S	S	8
10.	O.R.–8 ..	R	R	R	M	R	R	S	S	S	S	S	M	S	S	9
11.	Anakapalle ..	R	R	R	R	R	R	R	S	S	M	S	S	S	S	10
12.	West Bengal ..	R	R	R	R	R	R	M	S	S	S	S	S	S	S	11
13.	U.P.–13 ..	R	R	R	R	R	R	R	S	S	S	S	..	S	S	..
14.	Coochbehar ..	R	R	R	..	S	M	R	M	S	S	..	S	S	S	12
15.	O.R.–10 ..	R	R	R	..	M	..	R	M	M	S	..	S	S	S	..
16.	Madras -5 ..	..	R	R	..	R	R	R	R	R	..	..	S	S	S	..
17.	Srinagar ..	..	M	R	R	R	R	..	R	R	..	..	S	S	S	..
18.	Kalimpong ..	..	R	R	..	R	R	R	M	R	..	..	S	S	S	..
19.	Kanpur ..	R	R	R	..	M	M	M	M	S	S	..	S	S	S	..

R and M were taken as resistant.

The need for conducting artificial infection at temperature range of 20°–24° C. with high humidity is well understood and all techniques of artificial infection are based upon this knowledge.

The standardisation of procedures should go hand in hand with the development of an international set of differentials. Of the American differentials used, sharply differentiated reaction with the isolates studied so far in India could be obtained only with P.I. 180061, P.I. 201902, Wag Wag and Raminad Str. 3; the first two are from India and the latter two from the Philippines, all belonging to the *indica* group.

The varieties included from India have already shown differential reaction specially AC. 1613, Intan, CR. 906, SM. 6, Mas. and Bengawan. As has been shown in the test, U.S. race 25 could be further differentiated with some of the Indian types (Table IV).

Of the Japanese differentials only Ginga, Ishikari Shiroke, Yakeiko and Chokotou have given useful differential reaction.

An international set of differentials has to be developed amongst the above varieties and others proposed by Dr. Ou (personal communication) from the International Rice Research Institute, Manila, by Ahn and Chung (1962) from Korea and by Hung and Chien (1961) and Chien and Lin (1962) from Taiwan. A common set has been tentatively proposed further by the U.S. and Japanese workers (personal communication). These are being examined critically.

From the data presented it may be seen that certain varieties like B.J. 1, *S.* 67, *Zenith, Norin.* 22, *Te-tep* and *Tadukan* have proved resistant to all the identified races of India. This is indeed a very hopeful feature as the resistance of the varieties can be exploited in hybridization programmes to breed high yielding blast resistant varieties.

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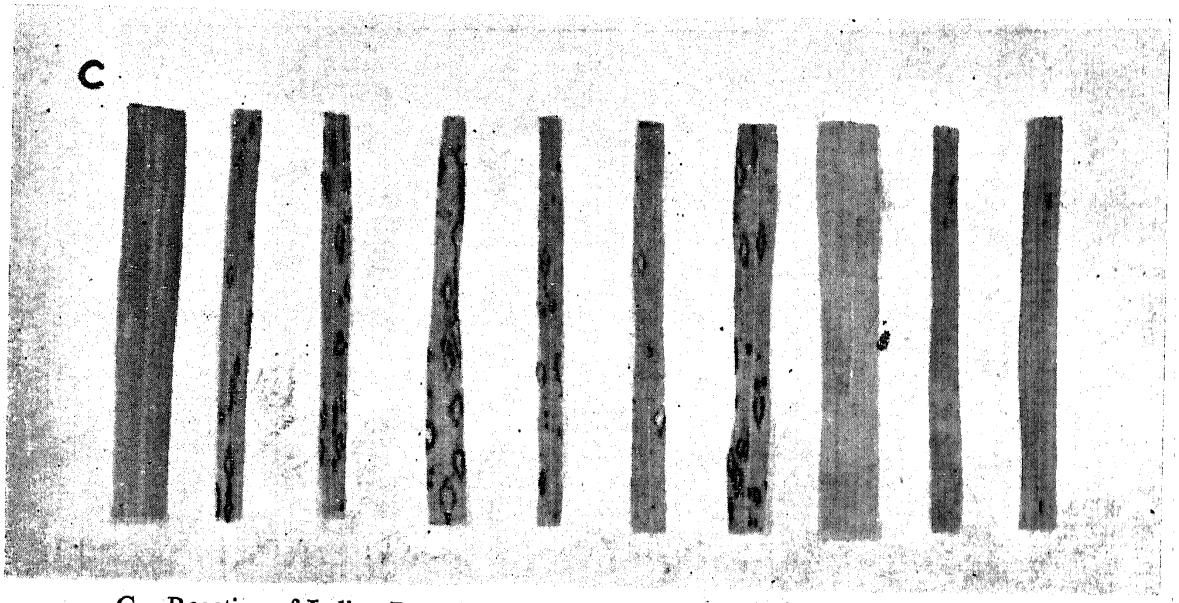
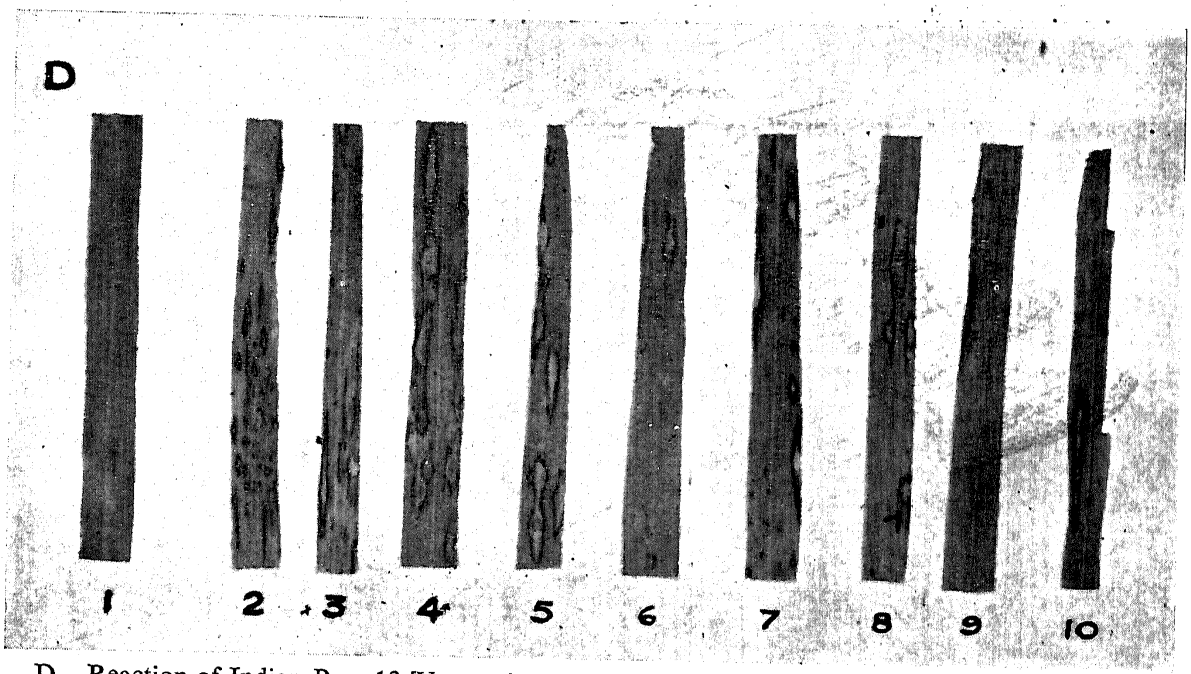
REFERENCEES

Ahn, C. J. and Chung, H. C. ... "Studies on the physiologic races of rice blast fungus, *Piricularia oryzae*, in Korea (in Korean, English Summary)," *Seoul Univ. J. Biol. and Agr.*, Series D, 1962, **11**, 77–83.

Seedlings of 34 differentials in nursery boxes.



C. Reaction of Indian Race 11 (Tripura isolate) on 10 U.S. differential varieties.



D. Reaction of Indian Race 13 [Varanasi (Uttar Pradesh) isolate] on 10 U.S. differential varieties.

Chien, C. C. and Lin, S. Y. .. "Physiologic races of the rice blast fungus," *Manuscript of paper from the Taiwan Agr. Res. Inst., Taipei, Taiwan,* 1962.

Goto, K. and Yamanaka, T. .. "Co-operative research on physiological races of *Piricularia oryzae,*" Spec. Rept. No. 5, *Studies on Insect and Disease Damage Spec.,* Suppl. No. 1, March 1961.

Hashioka, Y. .. "Studies on the mechanism of prevalence of rice blast disease in the tropics," *Taiwan Agr. Res. Inst. Tech. Bull.* 1950, **8,** 237.

Hung, C. S. and Chien, C. C. .. "Investigation of physiological races of the blast fungus," *Taiwan Agr. Res. Inst. Spec. Publ.,* 1961, **3,** 31–37.

Latterell, F. M., Tullis, E. C. and Collier, J. W. "Physiologic races of *Piricularia oryzae* Cav.," *Plant Dis. Reptr.,* 1960, **44** (9), 679–83.

——, Marchetti, M. A. and Grove, B. R. "Co-ordination of effort to establish an international system for race identification in *Piricularia oryzae.* The rice blast disease," *Proc. Symposium Int. Rice Res. Inst., July,* 1963. The John Hopkins Press, Baltimore, Maryland, 1965, pp. 257–74.

Padmanabhan, S. Y. .. "Physiologic specialization of *Piricularia oryzae* Cav. the causal organism of blast disease of rice," *Curr. Sci.,* 1964, **34** (10), 307–8.

—— and Ganguly, D. .. "Breeding rice varieties resistant to blast disease caused by *Piricularia oryzae.* 1. Selection of resistant varieties from genetic stock," *Proc. Ind. Acad. Sci.,* 1959, **50 B,** 289–304.

Richharia, R. H., Padmanabhan, S. Y., Veeraraghavan, J., Das, P. D. and Collaborators "Summary of results obtained under the co-operative blast resistance trials," *I.R.C. News Letter,* 1961, **10** (1), 8–13.

Suryanarayanan, S. .. "Mechanism of resistance of paddy (*Oryza sativa*) to *Piricularia oryzae* Cav. I. General considerations," *Proc. Natl. Inst. Sci. India, Biol. Sci.,* 1959, **24** (6) **B,** 285–92.