SPORULATION OF *PYRICULARIA* SPP. IN CULTURE. EFFECT OF SOME AROMATIC COMPOUNDS*

BY A. NARAYANARAO, K. MANIBHUSHANRAO AND S. SURYANARAYANAN, F.A.Sc.

(*University Botany Laboratory, Madras-5*)

Received September 18, 1971

ABSTRACT

The effect of L-tyrosine, DL-phenylalanine, shikimic acid and chlorogenic acid on sporulation was studied with six isolates of *Pyricularia* from different gramineous hosts. In general, the isolates from cultivated Gramineae sporulated better than the isolates from the wild grasses. The effect of the compounds on sporulation varied with the isolate. Possible genetic differences among the isolates are invoked to explain the results.

INTRODUCTION

THE potential for sporulation of *Pyricularia oryzae* Cav. on rice leaves has been shown to be maximum at the time when purplish brown pigment develops around lesions (Sasaki and Kato, 1963). The browning reaction around the lesions is believed to be due to polyphenols in rice tissues (Toyoda and Suzuki, 1953; Wakimoto *et al.*, 1960). Kato and Dimond (1966) reported that chlorogenic acid and L-tyrosine stimulated the production of conidia by *P. oryzae*. Since many of the phenolic constituents of higher plants appear to arise by further metabolism of aromatic amino acids or from intermediates involved in their biosynthesis (Neish, 1964), the effect of L-tyrosine, DL-phenylalanine and shikimic acid on sporulation of six isolates of *Pyricularia* was studied along with chlorogenic acid, the principal polyphenol of rice plants (Tamari *et al.*, 1963).

---

* Memoir No. 131 from the Centre for Advanced Study in Botany.

MATERIALS AND METHODS

Six isolates of *Pyricularia* as detailed below were used in the present study.

Isolate	*Natural host*	*Source*
1. *Pyricularia oryzae* Cav.	*Oryza sativa* L.	University Botany Lab., Madras-5.
2. *P. setariae* Nishikado	*Setaria italica* (Linn). P. Beauv.	Agriculture College and Research Institute, Coimbatore-3.
3. *Pyricularia* sp.	*Eleusine coracana* Linn.	do.
4. *Pyricularia* sp.	*Panicum repens* (Linn.) Derf.	C.R.R.I., Cuttack-6.
5. *Pyricularia* sp.	*Brachiaria mutica* (Forssk.) Stapf.	I.R.R.I., Manila, Philippines.
6. *Pyricularia* sp.	*Leersia hexandra* Swartz	do.

The stock cultures were maintained on potato-sucrose agar (2%).

The compounds were added before autoclaving to sucrose-nitrate medium (Suryanarayanan, 1958), so as to give the required final concentrations as indicated in the tables. The media were solidified with 2% agar. The initial pH of the media was adjusted to 6·0 with NaOH before sterilization. Each tube (15·0 × 1·5 cm.) had 5 ml. of the medium autoclaved at 15 lb/sq. in. for 15 min. Slants of uniform angle were made and inoculated at the centre with a pin-point inoculum from 10-day old stock cultures. The inoculated tubes were exposed to a bank of twelve 40 W (3,400 K) fluorescent tube lights at a distance of 20″ from the lamps in an air-conditioned growth chamber maintaining a temperature of 30±2°C during the light period and 20±1°C during the dark period. The tubes were exposed to light for a period of 12 h each day. Duplicate tubes were maintained for each treatment. Spore counts were made after 12 days incubation. Conidia were harvested by adding known amounts of water to the slants and gently disturbing the surface of

TABLE I

Sporulation of Pyricularia *spp. with L-tyrosine, DL-Phenylalanine and shikimic acid*

Substance	Concentration μg/ml.	Number of conidia/tube × 10^4					
		Isolates from					
		O. sativa	*E. coracana*	*S. italica*	*P. repens*	*B. mutica*	*L. hexandra*
L-tyrosine ..	0	210	45	52	3	0	20
	1	300	55	53	3	2	42
	6	395	38	71	7	*	18
	12	215	35	60	9	*	19
	18	200	32	53	8	*	18
	24	150	41	54	5	*	12
DL-phenylalanine ..	0	220	48	54	3	0	22
	1	150	45	45	4	*	16
	6	144	55	132	3	4	16
	12	125	35	99	5	2	34
	18	160	45	81	7	*	33
	24	250	84	82	4	*	45
Shikimic acid ..	0	215	47	50	3	0	21
	1	300	45	95	4	0	24
	6	315	100	145	10	*	38
	12	383	75	105	8	*	84
	18	330	60	105	5	2	72
	24	205	48	115	5	2	58

0: Nil sporulation

*: Sparse sporulation.

TABLE II

Sporulation of Pyricularia *spp. with chlorogenic acid*

Concentration µg/ml.	Number of conidia/tube × 10^4					
	Isolates from					
	O. sativa	*E. coracana*	*S. italica*	*P. repens*	*B. mutica*	*L. hexandra*
0 ..	190	44	70	6	0	10
50 ..	160	24	177	2	0	4
100 ..	220	36	215	5	0	2
200 ..	336	43	318	5	*	*
500 ..	615	95	510	4	*	*

0: Nil sporulation *: Sparse sporulation.

TABLE III

Sporulation of Pyricularia *sp. from* B. mutica *with high concentrations of chlorogenic acid*

Concentration µg/ml.	Number of conidia/ tube × 10^4
0	0
1000	9
2000	*
3000	0

0: Nil sporulation *: Sparse sporulation

the agar with a loop. The resulting suspension was thoroughly mixed and conidial numbers were estimated using a Thoma Haemocytometer. Ten separate counts were made for each slant.

Results and Discussion

The effect of L-tyrosine, DL-phenylalanine and shikimic acid on sporulation of six isolates of *Pyricularia* is shown in Table I and that of chlorogenic acid in Table II. It is evident from the results that, in general, the isolates from the cultivated cereals sporulated better than the isolates from the wild grasses. Among the isolates from the cultivated Gramineae, that for *E. coracana* sporulated less abundantly than those from *O. sativa* and *S. italica*. Similarly, the isolates from the grasses *P. repens* and *B. mutica* sporulated poorly as compared to the isolate from *L. hexandra*. In fact, the isolate from *B. mutica* sporulated only scantily in all treatments. These differences among the isolates may be ascribed to their innate genetic differences for sporulation.

Further support to the above view stems from the fact that the efficacy of the compounds in promoting maximum sporulation varied with the isolate. Thus, while chlorogenic acid promoted the best sporulation of the isolates from *O. sativa* and *S. italica*, the isolate from *E. coracana* responded equally well to the phenol or shikimic acid (Tables I and II). On the contrary, chlorogenic acid appeared to inhibit sporulation of the isolate from *L. hexandra*. It may also be seen that while the isolate from *O. sativa* sporulated better with tyrosine and shikimic acid than with phenylalanine, the isolates from *E. coracana* and *S. italica* sporulated better with shikimic acid and phenylalanine than with tyrosine. Such a difference between the two aromatic amino acids, however, was not evident in the case of the isolate from *L. hexandra*. These differences are possibly related to the phenylalanine/tyrosine-ammonia lyase systems of the isolates. Again, no marked differences were evident between the compounds on sporulation of the isolates from *P. repens* and *B. mutica*, except that only a high concentration of chlorogenic acid (1000 μ g/ml) had a small but significant effect on sporulation of the latter mentioned isolate (Table III). The effect of various phenolic compounds on sporulation of the six isolates of *Pyricularia* is presented in a subsequent communication.

Acknowledgements

We are grateful to Professor T. S. Sadasivan, Director, University Botany Laboratory, Madras-5, for his encouragement and interest in this problem.

One of us (A.N.) is thankful to the University Grants Commission, New Delhi, for the award of a fellowship during the tenure of which this work was carried out.

REFERENCES

Kato, H. and Dimond, A. E. .. "Factors affecting sporulation of the rice blast fungus, *Pyricularia oryzae*," *Phytopathology*, 1966, **56**, 864–65.

Neish, A. C. .. "Major pathways of biosynthesis of phenols," In *Biochemistry of Phenolic Compounds*. Academic Press, London and New York, 1964, pp. 295–359.

Sasaki, T. and Kato, H. .. "Source of inoculum for panicle blast in rice-potential for sporulation of lesions on leaves," *Phytopathol. Soc. Japan, Ann.*, 1963, **28**, 61–62.

Suryanarayanan, S. .. "Growth factor requirements of *Piricularia* spp. and *Sclerotium oryzae*," *Proc. Ind. Acad. Sci.*, 1958, **48 B**, 154–88.

Tamari, K., Ogaswara, N. and Kaji, J. "Biochemical products of the metabolism of *Piricularia oryzae*," In *The Rice Blast Disease*. John Hopkins Press, Baltimore, Maryland, 1963, pp. 35–68.

Toyoda, S. and Suzuki, N. .. "Histochemical studies on the lesions of rice blast caused by *Piricularia oryzae*—II", *Phytopathol. Soc. Japan, Ann.*, 1953, **17**, 97–101.

Wakimoto, S., Ikari, H. and Yoshii, H. "Relation between polyphenols contained in plants and phytopathogenic fungi (V)," *Kyushu Univ. Fac. Agr. Sci. Bull.*, 1960, **17**, 395–402.