Genetics of Stem Rot Resistance in Rice and Virulence in Sclerotium oryzae

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ABSTRACT

Comparison of 10 rice cultivars revealed different levels of resistance to stem rot disease. Progeny from crosses between cultivars differing in disease reaction were evaluated. Stem rot resistance was quantitatively inherited and transferable to a less resistant cultivar. Heritability of resistance in a Bluebelle × Colusa cross was 43%.

A large potential for variation in virulence among isolates of *S. oryzae* was observed. Single ascospore progeny of crosses of isolates differing in virulence were evaluated. Virulence in *S. oryzae* was quantitatively inherited and transgressive segregation of factors controlling virulence in the progeny was evident. In populations, there is a tendency for virulence to be stabilized at an intermediate level. Since the perfect stage of *S. oryzae* (*Magnaporthe salvinii*) can be found on diseased rice stems, this mechanism may be operable, with the effect that highly virulent isolates may not predominate in nature.

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Additional key words: disease resistance, virulence, *Magnaporthe salvinii.*

The literature on stem rot of rice caused by *Sclerotium oryzae* Catt. is scattered with reports of varying levels of disease resistance among different rice (*Oryza sativa* L.) cultivars (1, 2-4, 7-10, 13, 14, 18-20, 22-24, 26, 29). Although there is disagreement, most workers state that medium- to late-maturing, short grain, or tillering cultivars are more resistant than early maturing, medium or long grain, or nontillering cultivars (4, 7, 9, 10, 19, 21, 23-25, 27). Generally, the reports are sketchy concerning the experimental techniques and observations on which conclusions were drawn. Few studies, if any, have dealt with attempts to transfer the resistance or to determine the nature of its inheritance.

An impediment to progress in obtaining effective stem rot resistance was the lack of a method to estimate objectively the amount of disease on different rice cultivars. Recently, Krause and Webster (12) detailed such a method for field estimates of disease incidence and severity. This method should be useful for evaluating disease levels on mature plants in greenhouse inoculations. Rice cultivars could be compared; if differences exist, an analysis of the progeny of crosses between cultivars differing in resistance would yield estimates of the heritability of stem rot resistance.

Numerous examples can be found of the relative ease with which host resistance in the field is overcome by the pathogen (15, 16, 17, 28, 29). Therefore, in developing cultivars with lasting resistance in the field, knowledge of the potential for variation in virulence of the pathogen is desirable. Additionally, there is a need to understand the inheritance of resistance in the host and virulence in the pathogen. Then vertical or major gene resistance schemes involving multilines, gene deployment, and gene pyramiding can be employed effectively in plant disease control (16, 17). Knowledge concerning the inheritance of virulence as well as traits determining frequency and/or survival ability (fitness) of isolates differing in virulence are essential for developing a successful field cultivar using horizontal or minor gene resistance. Thus the genetic nature of host resistance to *S. oryzae* as well as virulence of the pathogen were studied.

MATERIALS AND METHODS.—*Rice cultivars*.—To determine availability of stem rot resistance, 10 rice cultivars ('Taichung 122', 'Tedoriwase', 'Norin 8', 'Norin 48', 'Italica Livorno', 'Szegedi Szakallas', 'Calrose', 'Colusa', 'Earlirose', and 'Bluebelle') were studied under greenhouse conditions. Szegedi Szakallas and Italica Livorno are very early maturing, bold medium grain cultivars, and Tedoriwase is a very early short grain rice. Norin 48, Taichung 122, and Colusa are early maturing, short grain cultivars, and Earlirose is early maturing but medium in grain length. Norin 8, Calrose, and Bluebelle are late maturing, short-, medium-, and long-grain rices, respectively. In addition, F_2 seed from a Calrose × Colusa and a Bluebelle × Colusa cross were used in studies to determine the complexity of inheritance of stem rot resistance.

Inheritance of stem rot resistance.—Plants were grown in Yolo loam soil in 5-liter pots lined with plastic bags, in the greenhouse under a 16-hour daylength regime. Plants, 7 weeks old, were inoculated by placing 150 mg of a mixture of sclerotia from six isolates of *S. oryzae* on the surface of water in the pots. These isolates differed in mating type and in ability to cause disease on the cultivar Calrose in greenhouse inoculations and in virulence as measured by the seedling test described previously (6).

TABLE 1. The stem-rot disease reaction of 10 rice cultivars after inoculation with a mixture of six isolates of *Sclerotium oryzae*

Cultivar	Number of plants	Disease index[a]	Standard deviation of the mean
Italica Livorno	25	4.71	.31
Tedoriwase	21	4.26	.56
Bluebelle	21	4.20	.25
Szegedi Szakallas	25	4.16	.50
Earlirose	25	3.75	.49
Calrose	11	3.71	.46
Norin 8	12	3.46	.22
Norin 48	11	3.38	.40
Taichung 122	21	3.24	.23
Colusa	25	3.13	.31

LSD ($P = 0.01$) = .29

[a]Disease index is that of Krause and Webster (12) which is based upon categories 1-5; (i) healthy, no symptoms or signs of disease; (ii) lightly infected, symptoms on outer leaf sheath only; (iii) mildly infected with discoloration of, and sclerotia in, the inner leaf sheath, culm green and healthy; (iv) moderately infected, slight to mild discoloration of the culm, interior of the culm healthy; (v) severely infected, culms infected internally, either collapsed or not.

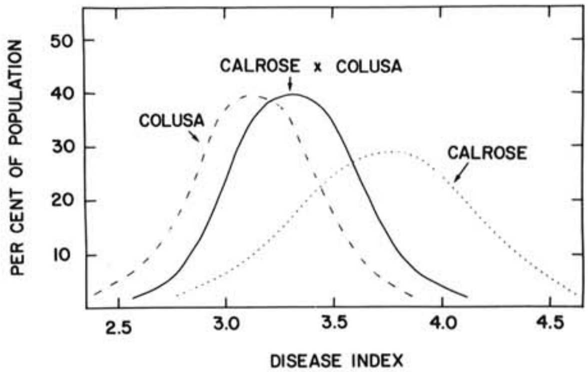

Fig. 1. The disease reaction of rice cultivars Colusa, Calrose, and the F_2-progeny from a cross between them, after inoculation with a mixture of six isolates of *Sclerotium oryzae*. The disease index of 25 Colusa plants was 3.13, with a standard deviation of the mean (s) of 0.31. The disease index of 103 F_2-progeny was 3.34, with an s value of 0.31. The disease index of 11 Calrose plants was 3.71, with an s value of 0.46. These statistical parameters were used to determine the frequency distribution of the disease reaction among the parental cultivars and their progeny.

The plants were fertilized once with $(NH_4)_2SO_4$ after they had been inoculated and were maintained under simulated flooded conditions with distilled water. Distilled water was used as the high boron content of tap water caused tip burn of rice. The rice was harvested after 20 weeks and the amount of disease was determined using the disease index of Krause and Webster (12).

Inheritance of virulence in S. oryzae.—*S. oryzae* is the sclerotial state of *Magnaporthe salvinii* (Cattaneo) Krause and Webster, a heterothallic ascomycete (11). Perithecia and ascospores are produced when isolates of appropriate mating type (A or a) are grown together on autoclaved rice stems on Sach's agar (24). To study the genetic nature of virulence in *S. oryzae*, progeny from crosses between isolates of the pathogen differing in virulence were tested for their virulence using a seedling test (6). The progeny from three different crosses were studied:

(i) D-30 × D-7 (high virulence × high virulence)
(ii) D-30 × D-34 (high virulence × low virulence)
(iii) D-15 × D-34 (low virulence × low virulence)

Single ascospore progeny from each cross were isolated from squashed perithecia. Sclerotia of the progeny were produced as described previously (6).

RESULTS.—*Inheritance of stem rot resistance*.—The differential disease reaction observed on 10 rice cultivars in greenhouse tests is indicated in Table 1. The most resistant cultivar studied was Colusa, and the most susceptible ones were Bluebelle, Earlirose, Italica Livorno, Szegedi Szakallas, and Tedoriwase. Rice cultivars differ in their photoperiod requirement for the induction of flowering (heading), thus photosensitive plants headed sparsely or late, if at all, under the 16-hour daylength regime, resulting in some excessive tillering. The prolonged vegetative phase probably resulted in underestimating the disease index (overestimating

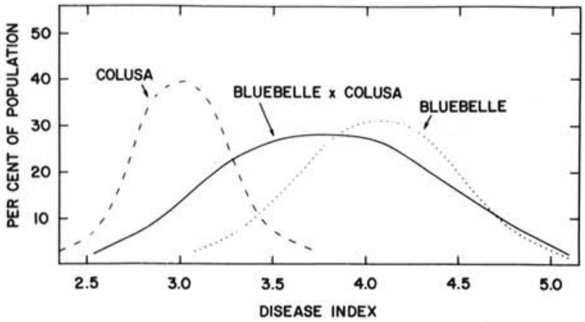

Fig. 2. The disease reaction of rice cultivars Colusa, Bluebelle, and the F_2 progeny from a cross between them, after inoculation with a mixture of 10 isolates of *Sclerotium oryzae*. The disease index of 35 Colusa plants was 3.03, with a standard deviation of the mean (s) of 0.31. The disease index of 128 F_2-progeny was 3.77, with an s value of 0.61. The disease index of 45 Bluebelle plants was 4.04, with an s value of 0.57. These statistical parameters were used to determine the frequency distribution of the disease reaction among the parental cultivars and their progeny.

resistance) in Calrose, Norin 8, and Norin 48. Italica Livorno, Szegedi Szakallas, Taichung 122, and Tedoriwase were early mating cultivars and may have been rated too late. If so, estimates of resistance may be low. Nevertheless, significant differences were noted between Colusa, Earlirose, and Bluebelle for which the photoperiod was suitable.

The disease reaction of the Calrose × Colusa progeny is given in Fig. 1. The photoperiod effect on Calrose, and some of the progeny made it difficult to estimate the disease index for these plants at a uniform stage of maturity. As a result, heritability estimates for stem rot resistance were not calculated. The results, however, do indicate that stem rot resistance is continuously variable and quantitatively inherited, because no discrete groups or classes were evident from this cross.

Colusa and Bluebelle were similar in photoperiod response, yet differed greatly in their susceptibility to *S. oryzae*, so F_2 progeny from a Bluebelle × Colusa cross were studied. The procedure for testing the progeny and parents for their disease reaction was identical to the previous Calrose × Colusa cross with the exception that the mixture of sclerotia used as inoculum consisted of 10 different isolates of the pathogen. The disease reaction of both the parents and the progeny is given in Fig. 2. Differences between the parental cultivars and the progeny of a cross between them were significant, $P = 0.01$. As in the case of the Calrose × Colusa cross, stem rot resistance was quantitatively inherited. The variances (σ^2) of the parental cultivars were used to estimate the environmental component of variation and the heritability (H) of resistance was estimated as 43% using the formula:

$$H = \frac{\sigma^2_{\text{Progeny}} - 1/2(\sigma^2_{\text{Colusa}} + \sigma^2_{\text{Bluebelle}})}{\sigma^2_{\text{Progeny}}}$$

Inheritance of virulence in S. oryzae.—The data indicating the mode of inheritance for virulence in *S. oryzae* are shown in Fig. 3. The mean virulence for the progeny of each of the crosses was intermediate to that of the parental isolates. The distribution of virulence among the progeny of each cross indicated that virulence in *S. oryzae* is continuously variable and inherited quantitatively.

Whenever a highly virulent isolate was involved in the cross, transgressive segregation occurred so that some of the progeny were less virulent than either parent. In the cross between the two low-virulence isolates, transgressive segregation occurred which caused some of the progeny to be either more or less virulent than each parent.

DISCUSSION.—Although stem rot resistance was quantitatively inherited, the heritability of resistance was estimated to be 43%, indicating progress toward developing agronomically desirable cultivars resistant to stem rot should be possible. A breeding program would involve examining large numbers of progeny from crosses involving cultivars with the desired traits. A method to quickly evaluate progeny for disease resistance has been described (6).

The most resistant cultivar in greenhouse tests was Colusa, confirming field observations that Colusa is the most resistant of the commercially grown Californian cultivars (12). Colusa might serve as a source of resistance to stem rot in improving the desirable medium or long

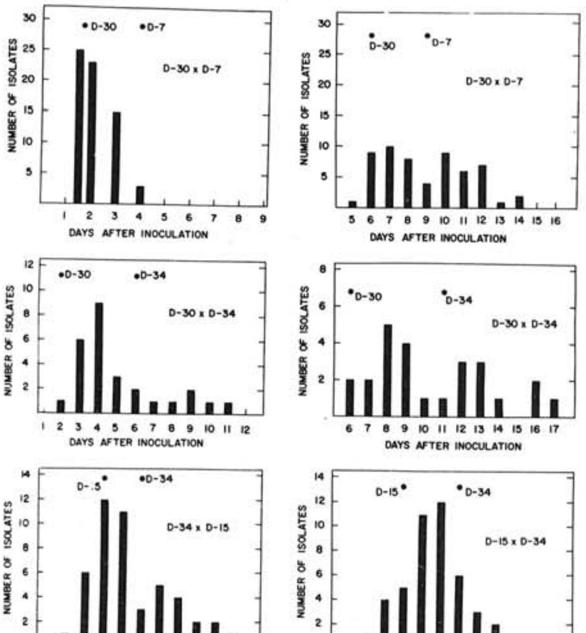

Fig. 3. The inheritance of virulence among single ascospore progeny for three crosses of *Sclerotium oryzae*. The crosses involved two high-virulence isolates (D-30 and D-7), a high- and a low-virulence isolate (D-30 and D-34, respectively), and two low-virulence isolates (D-15 and D-34). Virulence was measured using a seedling test to measure the time in days for half of 10 inoculated 25-day-old Colusa seedlings to be infected ($LR_{1/2}$) or killed ($KR_{1/2}$). The virulence of the parental isolates are indicated by solid dots.

grain rices such as Calrose or Bluebelle. Other rice cultivars and related wild species should be studied and evaluated as alternative sources of stem rot resistance which may prove to be better than that found in Colusa.

As with resistance to stem rot in rice, virulence in the pathogen is quantitatively inherited; the mean virulence of the progeny was intermediate to that of the parental isolates, and no discrete groups or classes of virulence were found among the progeny. The occurrence of transgressive segregation among progeny of crosses of *S. oryzae* which differed in virulence indicates that within populations of *S. oryzae* there may be a tendency toward an intermediate level of virulence. Quantitatively inherited traits displaying an intermediate tendency have been described by population geneticists to be under the influence of stabilizing selection (5). Van der Plank (28) has proposed that in the case of horizontal resistance, stabilizing selection operates on the pathogen so that highly virulent isolates of the pathogen are less fit, and therefore are not likely to predominate in the population and overcome horizontally resistant hosts. This situation may exist for *S. oryzae*.

Because stem rot resistance is quantitatively inherited, rice might be considered horizontally resistant in van der Plank's (28, 29) concept of the term. Because all isolates displayed some (but different) abilities to cause disease on the cultivar Colusa, it was not uniformly resistant to all isolates of the pathogen. For this reason, Colusa may not be considered horizontally resistant. However, it is not likely that differentiation of isolates of the pathogen for their disease reaction on Colusa was due to the action of vertical or major genes, because virulence in *S. oryzae* was shown to be inherited quantitatively. It appears that the terms "horizontal resistance" and "vertical resistance", as defined by van der Plank (28, 29), may not be applicable to stem rot resistance in rice.

LITERATURE CITED

1. ADAIR, C. R., and E. M. CRALLEY. 1950. 1949 rice yield and disease control tests. Arkansas Agric. Exp. Stn. Rep. Ser. 15. 20 p.
2. CALIFORNIA COOPERATIVE RICE RESEARCH FOUNDATION. 1973. Rice breeding program progress report. 10 p.
3. CALIFORNIA COOPERATIVE RICE RESEARCH FOUNDATION. 1974. Rice breeding program progress report. 23 p.
4. CRALLEY, E. M. 1936. Resistance of rice varieties to stem rot. Arkansas Agric. Exp. Stn. Bull. 329. 31 p.
5. CROW, J. F., and M. KIMURA. 1970. An introduction to population genetics theory. Harper and Row. New York, Evanston, and London. 591 p.
6. FERREIRA, S. A., and R. K. WEBSTER. 1974. A method to evaluate the virulence in isolates of Sclerotium oryzae. Phytopathology 64:972-976.
7. GOTO, K., and R. FUKATSU. 1954. Studies on the stem rot of rice plant. I. Varietal resistance and seasonal development. Div. Plant Breed. Cult., Tokai-Kinki Nat. Agric. Exp. Stn. Bull. 1:27-39. (In Japanese, English summary).
8. HECTOR, G. P. 1931. Annual report of the First Economic Botanist to the Government of Bengal for the year 1930-31. Rep. Dept. Agric. Bengal, 1930-31:34-44. [Rev. Appl. Mycol. 11:158, (1932)].
9. IRRI (INTERNATIONAL RICE RESEARCH INSTITUTE). 1965. Pages 112-114 *in* Annual report. Los Banos, Philippines.
10. IRRI (INTERNATIONAL RICE RESEARCH INSTITUTE). 1966. Pages 89-90 *in* Annual report. Los Banos, Philippines.
11. KRAUSE, R. A., and R. K. WEBSTER. 1972. The morphology, taxonomy, and sexuality of the rice stem rot fungus, Magnaporthe salvinii (Leptosphaeria salvinii). Mycologia 64:103-114.
12. KRAUSE, R., and R. K. WEBSTER. 1973. Stem rot of rice in California. Phytopathology 63:518-523.
13. LO, T. C., and S. P. Y. HSIEH. 1964. Studies on the stem rot of rice plant. I. Distribution and economic significance of the disease. Plant Prot. Bull., Taiwan 6:121-135.
14. LUTHRA, J. C., and A. SATTAR. 1936. Some studies on the sclerotial diseases of rice (Sclerotium oryzae Catt.) in the Punjab. Indian J. Agric. Sci. 6:973-984.
15. NATIONAL RESEARCH COUNCIL COMMITTEE ON GENETIC VULNERABILITY OF MAJOR CROPS. 1972. Genetic vulnerability of major crops. National Academy of Sciences, Washington, D.C. 307 p.
16. NELSON, R. R. 1972. Stabilizing racial populations of plant pathogens by use of resistance genes. J. Environ. Qual. 1:220-227.
17. NELSON, R. R. 1973. Breeding plants for disease resistance—concepts and applications. Pennsylvania State University Press. University Park and London. 401 p.
18. NONAKA, F., and H. YOSHII. 1958. Relationship between the maturity of rice plant and the severity of stem rot caused by Leptosphaeria salvinii Catt. I. Seasonal observations of the severity and culm invasion of the causal fungus. Sci. Bull. Fac. Agric. Kyushu Univ. 16:439-445. (In Japanese with English summary).
19. ONO, K. 1949. Varietal resistance to stem rot in rice. Annu. Phytopathol. Soc. Jap. 13:14-18. (In Japanese).
20. ONO, K., and H. SUZUKI. 1960. Studies on mechanism of infection and ecology of blast and stem rot of rice plant. Spec. Rep. Forecast. Disease Insect Pest. 4:94-152. Minist. Agric. For. Fish., Japan. (In Japanese with English summary).
21. OU, S. H. 1972. Rice diseases. Commonwealth Mycological Institute. Kew, Surrey, England. 368 p.
22. PARACER, C. S., and J. C. LUTHRA. 1944. Further studies on the stem rot disease of rice caused by Sclerotium oryzae Catt. in the Punjab. Indian J. Agric. Sci. 29:44-48.
23. REYES, G. M. 1929. A preliminary report on the stem rot of rice. Philippine Agric. Rev. 22:313-331.
24. REYES, G. M. 1936. Rice hybrids versus stem rot disease. Philippine J. Agric. 7:413-418.
25. SHOEMAKER, R. A. 1955. Biology, cytology, and taxonomy of Cochliobolus sativus. Can. J. Bot. 33:562-576.
26. TISDALE, W. H. 1921. Two sclerotial diseases of rice. J. Agric. Res. 21:649-658.
27. TULLIS, E. C., and E. M. CRALLEY. 1933. Laboratory and field studies and the development and control of stem rot of rice. Bull. Arkansas Agric. Exp. Stn. 295:3-22.
28. VAN DER PLANK, J. E. 1963. Plant diseases: epidemics and control. Academic Press, New York and London. 349 p.
29. VAN DER PLANK, J. E. 1968. Disease resistance in plants. Academic Press, New York and London. 206 p.