Seed Germinability Tests for Predicting Field Emergence of Rice Seeds Infected with Helminthosporium oryzae and Trichoconis padwickii

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ABSTRACT

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Forty-eight lots of rice seeds were evaluated by the blotter health test for the amount of infection by *Helminthosporium oryzae* and *Trichoconis padwickii*. Germinability of these seed lots kept in towels in a germinator, in sand at two depths in the greenhouse, and in soil in a growth chamber, was determined at several temperatures. Twenty-six lots were sown in the field and seedling emergence was recorded. Infection of seeds by *H. oryzae* was closely correlated (r = −0.82**) with seedling emergence in the field. In contrast, infection by *T. padwickii* correlated poorly (r = 0.09) with field emergence. Highly significant correlation coefficients were obtained between field emergence and germinability in (i) towels at 15, 20, and 30 C, (ii) in the sand bench under 3 cm of sand and cool conditions, and (iii) in soil in a growth chamber at 20 C. Infection by *H. oryzae* was closely correlated ($P = 0.01$) with the results of these same three tests, with the exception of the towel test at 30 C. Thus, the towel test at 15 or 20 C can substitute for the blotter health test to predict the field emergence of *H. oryzae*-infected rice seeds. The correlation between *H. oryzae* infection and germinability in sand at 3 cm in a cool greenhouse was not so good as with the other tests. The soil test in a growth chamber at 20 C can predict field emergence of rice seeds, but it is not practical for routine work.

The agar and blotter health tests are the two principal types of methods currently used for the routine detection of pathogenic fungi in crop seeds (6). However, these two techniques are time-consuming, require personnel with training in mycology for evaluation of the results, and they usually require a compound microscope and blacklight-equipped incubators. These drawbacks may be partially responsible for the virtual absence of routine seed health testing in seed-testing laboratories in the USA.

There is now a biochemical method for detecting *H. oryzae* and *T. padwickii,* the two most common pathogenic fungi found in rice seeds produced in the USA (4). Although this new method has none of the drawbacks of the agar or blotter methods of seed health testing, it probably will be some time before it is widely used. In the interim, it may be possible to modify and substitute for the agar or blotter health tests techniques such as the towel test now used by seed testing laboratories in the USA for the routine determination of seed germinability. This substitution could be valid if the results of a germinability test correlated as well with field emergence data as did the results from an agar or blotter health test done on the same seed lots.

In the present study, seeds of rice (*Oryza sativa* L. 'Starbonnet') were assayed on blotters for infection by *Helminthosporium oryzae* Br. de Haan [syn. *Drechslera oryzae* (Br. de Haan) Subr. & Jain; *Bipolaris oryzae* (Br. de Haan) Shoemaker] and *Trichoconis padwickii* Ganguly [syn. *Alternaria padwickii* (Ganguly) M. B. Ellis]. In addition, the same seed lots were assayed for germinability by (i) the towel test, (ii) in a greenhouse sand bench, and (iii) in flats of soil in a growth chamber. The percent seedling emergence of more than half of these lots also was determined in the field.

MATERIALS AND METHODS

Forty-eight lots of seeds harvested in the southeastern U.S. were used in the laboratory and greenhouse studies; 26 of these were sown in the field study.

Determination of seed-borne pathogens.—The modified blotter health test reported in an earlier paper was used in this study (4).

Germinability and seedling emergence tests.—Two-hundred seeds per lot were used in each test except the field test. In the towel test, seeds were germinated between moist, folded paper towels kept for 14 days in a germinator at 15, 20, 25, 30, or 35 C. This test was evaluated according to the rules of the Association of Official Seed Analysts (1); i.e., a seed was considered to be germinated when it had produced a normal root and shoot. Seeds also were sown in a greenhouse bench filled with steamed (100 C for 6 hr) sand, and covered 3 or 9 cm deep. The greenhouse was kept relatively cool (avg 15-17 C at night, and 23-25 C during the day), or relatively warm (avg 23-25 C at night, and 30-33 C during the day). The number of seedlings that emerged from 3 cm under cool or warm conditions was recorded after 22 days, and from 9 cm at 24 and 27 days for warm and cool conditions, respectively. Seeds sown 3 cm deep in flats of steamed soil were kept in a growth chamber at 20 or 30 C for 28 and 16 days, respectively. In the field test, 1,500 seeds from each of 26 lots were sown 3 cm deep in a completely randomized block consisting of four replications (375 seeds per replication). The average soil and air temperatures at 1000 hours during the period of

TABLE 1. Correlation coefficients among percentage infection for *Helminthosporium oryzae* (HO) and *Trichoconis padwickii* (TP), and percentage germinability and seedling emergence in 48[a] lots of rice seeds tested under different conditions

Factor	Germinability or emergence per test condition (%)											
	In towels					Sand-GH[b] 3 cm deep		Sand-GH[b] 9 cm deep		Soil-GC[c]		Field[d]
	15 C	20 C	25 C	30C	35 C	Cool	Warm	Cool	Warm	20 C	30 C	
% Seed-borne infection[e]												
HO	−.69**[f]	−.57**	.02	−.06	.17	−.52**	.10	−.30*	−.10	−.55**	.02	−.82**
TP	.34*	.28	.17	.33*	.27	.56**	.18	−.35*	−.31*	.38	.38**	.09
% Field emergence	.78**	.79**	.47*	.70**	.36	.58**	.01	.42*	.30	.74**	.14	1.00

[a]Of which 26 lots were in the field.
[b]GH = greenhouse.
[c]GC = growth chamber.
[d]Average soil and air temperatures at 10 A.M. during the period of this test were 16-18 C and 18-22 C, respectively.
[e]Determined in a blotter health test.
[f]Asterisks: * = significant at $P = 0.05$, and ** = significant at $P = 0.01$.

this test were 16-18 C and 18-22 C, respectively. Counts of emerged seedlings were made 21 days after sowing.

The data obtained in each test were paired with the data from each of the other tests and correlation coefficients (r) were calculated.

RESULTS AND DISCUSSION

Infection of rice seeds by *H. oryzae,* as determined in the blotter health test, was negatively correlated (r = −0.82**) with seedling emergence in the field (Table 1). These results are in general agreement with those observed by Aulakh et al. (2) in a study of eight samples found in a blotter health test to have between 9 and 49% of *H. oryzae*-infected seeds. They sowed seeds from their samples in pots of sterilized soil kept at 21-27 C and found that the ratio between infected seeds and infected seedlings was 1.0:0.8.

Under the test conditions reported here, infection of seeds by *T. padwickii* correlated poorly (r = 0.09) with field emergence, indicating that this fungus did not have an adverse effect on rice seed germinability. Our results differ from those reported by Cheeran and Raj (3) and Mathur et al. (5). These differences may have arisen because (i) our test conditions differed from theirs, (ii) climatic conditions in the southeastern U.S. are not suitable for severe infection of rice seeds by *T. padwickii,* (iii) there are differences in virulence between fungal strains, or (iv) Starbonnet rice may be less susceptible to infection by *T. padwickii* than the cultivars they used.

The correlation coefficients were highly significant between field emergence and germinability in the towel test at 15, 20, and 30 C, in the sand bench test under cool conditions with the seeds under 3 cm of sand, and in soil in a growth chamber at 20 C. Percentage infection by *H. oryzae* correlated very closely (r = −0.52** or greater) with germinability in each of these three tests, with the exception of the towel test at 30 C (r = −0.06, Table 1).

It appears that the towel test at 15 or 20 C can be substituted for the blotter health test to predict the emergence capability of *H. oryzae*-infected rice seeds. Although the correlation between emergence in the field and germinability in sand at 3 cm in a cool greenhouse was highly significant (r = 0.58**), its magnitude was lower than the other highly significant correlations (r = 0.70** or greater, Table 1). For this reason, the sand test may not be a reliable substitute for the blotter health test of *H. oryzae*-infected rice seeds.

Results from soil in the growth chamber at 20 C also were highly significant. Considering the cost of this apparatus, however, it is unlikely that this test would be used routinely as a substitute for the blotter health test.

Although a highly significant correlation (r = 0.70**) was obtained between germinability in towels at 30 C and percent field emergence, percentage infection by *H. oryzae* correlated very poorly (r = −0.06) with towel germinability at 30 C. This plus Ocfemia's observation (7) that the greatest amount of preemergence infection by *H. oryzae* occurs at 16-24 C, suggest that the towel test at 30 C may not be a reliable substitute for the blotter health test for predicting the field emergence of *H. oryzae*-infected rice seeds.

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