Effect of Light, pH and Calcium Chloride on Seed-Borne *Sarocladium oryzae* in Rice

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SUMMARY

Adjusting the pH of water to 6.5 before incubating rice seeds in blotter test recorded maximum *S. oryzae* seed infection count in all the six seed samples tested. The maximum per cent *S. oryzae* seed infection count (48.67%) was observed in the case of variety ADTRH 1 followed by CORH 2 (39.67%) at pH 6.5. The blotters dipped in pH 8 revealed minimum seed infection count of *S.oryzae* irrespective of the seed samples tested as against pH 6.5. The results indicated that pH of water played an important role on the *S.oryzae* seed infection count. The maximum *S.oryzae* infection count was recorded when the seeds were exposed to 12 hrs NUV + 12 hrs darkness followed by 8 hrs NUV+ 16 hrs darkness in both the samples tested. ADTRH 1 recorded maximum *S.oryzae* (47%) at 12 hrs NUV + 12 hrs darkness followed by 8 hrs NUV + 16 hrs darkness (44.33%) on 8th day of incubation. In general, soaking the seeds in different concentrations of $CaCl_2$ increased *S. oryzae* seed infection count in all the rice seed samples tested. Among the different concentrations of $CaCl_2$ tested, soaking the seeds in 2 and 3 per cent concentration recorded more *S.oryzae* seed infection count than other concentrations tested.

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Rice (*Oryza sativa* L.) seeds are known to harbour a wide range of both fungi and bacteria (Neergaard, 1977). A total of more than 100 fungi have been detected on rice seeds. About 20 species of fungal pathogens were detected from rice seed at any one time (Mew and Gonzales, 2002). Among them, sheath rot caused by *Sarocladium oryzae* is an important pathogen mainly affecting the economic part of the rice plant. The fungus is detected frequently during routine seed health testing.

Several methods have been evolved for detection of seed-borne microorganisms which have been reviewed from time to time (De Tempe, 1961, 1963, 1964; Agarwal, 1976; Neergaard, 1977; Agarwal and Sinclair, 1987; Gaur and Dev, 1988; Khare, 1996). The purpose of seeds health testing is to assure the safe movement of seed of different crops, for research or trade. It can also be a means of quality control to improve seeding stocks for crop production by farmers. Many detection methods have been developed over the years for various seed borne pathogens. Several criteria have to be considered in selecting a suitable routine seed health testing procedure. Primarily, it should be capable of revealing maximum pathogen infection and should also be versatile, revealing a range of pathogens (Singh *et al*., 1974).

The blotter and guaiacol agar methods were compared for the detection of *Helminthosporium oryzae* and *Trichoconis padwickii* in rice seeds. Guaiacol agar method appeared to be more sensitive than the Blotter method for the detection of *H. oryzae*, and less sensitive for the detection of *T. padwickii*. The Guaiacol agar method was much faster than the blotter method and did not require a microscope and black light equipped incubator (Kulik, 1975). Mathur (1979) recommended Potato dextrose agar for recording *T. padwickii* in rice seed lots. Paddy seeds were soaked in 0.2 per cent NaOH for 24 hours and incubated at 18 – 25° C. Little use has been made of serological methods for the detection of fungi, particularly in seed. Walez *et al*. (1985) showed that the enzyme-linked immunosorbent assay (ELISA) could be used to detect antigen of *Sclerotinia sclerotiorum* (Lib.) de Bary at a concentration as low as 10 ng $ml^{-1.}$ However, this fungus can be detected in seed samples by simple direct examination. Mia *et al*. (1985) found that standard blotter and deep freezing methods were equally effective in recording seed-borne infection of *Gerlachia oryzae*.

Shetty and Shetty (1988) developed Rice extract agar and its efficacy was compared with five other methods *viz*., standard blotter, 2,4- D, Deep-freezing blotter, Potato dextrose agar and Guaiacol agar. Rice extract agar was

equal to all methods other than Potato dextrose agar in revealing the per cent seed infection by *T. padwickii*. However, rice extract agar is preferred because it allows the macroscopic identification of the fungus. There was no significant difference in the incidence of *Drechslera oryzae, Fusarium moniliforme* and *Rhynchosporium oryzae* in these six methods.

An interesting application of the osmotic principle in seed health testing was developed by Brodal (1997) to detect *Drechslera* species in cereal seeds. The incorporation of sucrose in solution used to moisten blotters provided conditions for the *Drechslera* species to change the colour of the substrate to pink-violet at the contact with incubated seeds. Modern molecular methods for characterization and diagnosis of seed- borne fungal pathogens offers the potential for sensitive and specific detection of pathogens and for detection of more than one pathogen in a single test by multiplex PCR and/or the use of fluorescent probes or primers. These methods are also suitable for automation and are thus more time and cost-effective than conventional methods. However, only a limited number of these have been developed into seed health tests. This may be partly due to technical difficulties, cost of PCR license and royalties as well as the time and organization needed for test standardization (Taylor *et al*., 2001).

Due to germination of seeds in Blotter method, the seed coats are lifted at different levels which make the seed examination for associated fungi difficult. To overcome this problem, the blotters are dipped in 0.1 to 0.2 per cent solution of sodium salt of 2,4 – dichlorophenoxyacetic acid in water. Neergaard and Saad (1962) observed higher percentage of *Pyricularia oryzae* in paddy seeds using 2,4 – D method. *Alternaria dauci* was detected in carrot seeds when incubated on filter paper moistened with 2,4–D amended water or carrot leaf extract. Concentrations of 2,4-D greater than 0.1 per cent inhibited growth and sporulation of *A. dauci*. But concentration between 0.0001 and 0.01 per cent had no significant effects on the pathogen or efficiency of detection (Strandberg, 1988). Shukla *et al*.(1990) also reported higher counts of *Phoma medicaginis* f.sp. *sojae* Sacc. from soybean seeds. In another modification, the blotter papers were dipped in water and the seeds were pretreated with 2, 4 – D solution. Since, *S.oryzae* is a slow growing pathogen on rice seeds, it is often suppressed by the over growth of other seed borne fungi like *Alternaria* and *Bipoloris.* Hence, the present study was conducted to assess the effect of light sources, pH and Calcium chloride on *S.oryzae* detection in blotter testes, so that a new approach can be formulated to modify (or) improve the existing method to pave for easy detection of *S.oryzae* in rice seeds.

MATERIALS AND METHODS

Effect of different pH levels on the detection of S.oryzae in rice seeds:

Three layers of blotters were dipped in sterilized water with different pH levels *viz*., 5.0, 6.0, 6.5, 7.0, 7.5 and 8.0 separately and placed in the Petri plate after draining off excess water. Rice seeds with different levels of seed infection were placed at equi-distance in each Petri plate @ 25 seeds per plate.

Effect of different light sources on the infection counts of S.oryzae:

Near Ultra Violet (NUV) light source was provided by Philips T1 40W/08 black light tubes and artificial day light (ADL) by Philips TLF 40W/34 white cool fluorescent tubes. The tubes mounted in pairs, were placed at a distance of 41 cm above the seeds. For obtaining complete darkness the, Petri dishes were wrapped in thick black paper. Different combinations of light and darkness were tested. Seeds of two cultivars with *S.oryzae* seed infection namely, ADTRH 1 (47%) and ADT 39 (33%) were used in the study and for each treatment 400 seeds were examined. The seeds were incubated at 4 hrs NUV + 20 hrs darkness, 8hrs NUV + 16 hrs darkness, 12 hrs NUV + 12 hrs darkness and complete darkness. Twenty five seeds were placed on moist blotters in each Petri dish and were incubated for six and eight days, respectively at 20 ± 1° C and examined for *S.oryzae* seed infection.

Calcium chloride method:

The rice seeds were soaked in different concentrations of calcium chloride *viz*., 1, 2, 3, 5 and 8 per cent overnight and the soaked rice seeds of different varieties were placed at equi- distance in each Petri plate @ 25 seeds per plate and incubated as per standard blotter method (Renukeswarappa and Sethna, 1985).

RESULTS AND DISCUSSION

Effect of pH level on the detection of S.oryzae:

The effect of six different levels of pH *viz*., 5.0, 6.0, 6.5, 7.0, 7.5 and 8.0 of water used to moisten the blotter discs was studied with six rice seed samples having *S. oryzae* seed infection and the results are furnished in the Table 1. Adjusting the water to pH 6.5 recorded maximum seed infection count in all the six seed samples tested. The maximum per cent *S. oryzae* seed infection count (48.67%) was observed in the case of ADTRH 1 followed by CORH2 (39.67%) at pH 6.5. The blotters dipped in

Table 1 : Effect of different pH levels on the detection of *S.oryzae*

Sr. No.	pH	*S. oryzae* infected seeds* (%)					
		IWP	ASD16	ADT36	IR50	ADTRH1	CORH2
1.	5.0	24.33 [a]	8.00 [a]	27.67 [b]	6.00 [ab]	31.00 [ab]	32.33 [ab]
		(29.54)	(16.41)	(31.72)	(14.07)	(33.83)	(34.58)
2.	6.0	27.67 [ab]	14.00 [b]	33.33 [c]	6.33 [b]	38.00 [ab]	39.67 [b]
		(31.72)	(21.95)	(35.25)	(14.51)	(38.05)	(39.03)
3.	6.5	29.67 [b]	17.00 [b]	35.33 [c]	9.33 [b]	48.67 [b]	39.67 [b]
		(32.99)	(24.30)	(36.46)	(17.75)	(44.32)	(39.03)
4.	7.0	24.33 [a]	15.33 [b]	31.33 [bc]	7.67 [b]	35.33 [ab]	32.67 [ab]
		(29.54)	(22.93)	(34.02)	(15.72)	(36.43)	(34.80)
5.	7.5	25.33 [ab]	13.33 [b]	27.00 [b]	5.33 [ab]	24.33 [a]	27.35 [a]
		(30.21)	(21.27)	(31.28)	(13.11)	(29.23)	(31.48)
6.	8.0	23.33 [a]	12.33 [ab]	19.67 [a]	2.67 [a]	22.33 [a]	24.33 [a]
		(28.86)	(20.47)	(26.31)	(9.27)	(28.11)	(29.50)

* Mean of 3 replications; 200 seeds per replication. Figures in parentheses are arc sine transformed values.
Means followed by a common letter are not significantly different at 5% level by DMRT

pH 8 revealed minimum seed infection count of *S.oryzae* irrespective of the seed samples tested as against pH 6.5. The results indicate that pH of water plays an important role on the seed infection count. The optimum pH of 6.5 resulted in maximum *S.oryzae* seed infection count in all the seed samples. pH 5 and 8 were found unfavourable for *S.oryzae* growth and sporulation. Not much work related to the influence of pH on *S.oryzae* is available. However, the influence of pH on growth and sporulation varied between the organisms found associated with seeds. Singh *et al*. (1982) found that pH 4 gave higher counts of *A. padwickii* associated with paddy seeds.

Effect of light on the detection of S.oryzae:

In all the treatments, 8 days of incubation yielded more *S.oryzae* than 6 days of incubation irrespective of the samples tested (Table 2). The maximum *S.oryzae* infection count was recorded when the seeds were exposed to 12 hrs NUV + 12 hrs darkness followed by 8 hrsNUV+ 16 hrs darkness in both the samples tested. ADTRH 1 recorded maximum *S.oryzae* (47%) at 12 hrs NUV + 12 hrs darkness followed by 8 hrs NUV + 16 hrs darkness (44.33%) on 8th day of incubation. In ADT 39 also, 12 hrs NUV + 12 hrs darkness recorded maximum *S.oryzae* (34.67%) followed by 8 hrs NUV + 16 hrs darkness (33.00%) on 8th day of incubation. The least count was recorded when the seeds were incubated in complete darkness in both the seed samples. The present study indicated that 8 days of incubation provided with 12 hours NUV + 12 hours darkness exposure yielded more *S.oryzae* seed infection count. The present result was similar to the findings of Chang *et al*. (1972) who observed that irradiation with NUV or artificial day light is an essential factor for incubation of most seed-borne fungi of rice in the blotter test.

Table 2 : Effect of Near Ultra Violet (NUV) on infection counts of *S.oryzae*

Sr. No.	Treatment	*S. oryzae* infection (%)*			
		ADTRH 1		ADT 39	
		6th day	8th day	6th day	8th day
1.	4 hours NUV +	34.33 [b]	40.33 [b]	28.00 [ab]	30.33 [ab]
	20 hours darkness	(35.82)	(39.42)	(31.93)	(33.39)
2.	8 hours NUV +	38.67 [b]	44.33 [b]	30.33 [ab]	33.00 [b]
	16 hours darkness	(38.43)	(41.74)	(33.41)	(35.05)
3.	12 hours NUV +	41.00 [b]	47.00 [b]	31.67 [b]	34.67 [b]
	12 hours darkness	(39.79)	(43.28)	(34.21)	(36.03)
4.	Complete darkness	18.67 [a]	24.67 [a]	23.33 [a]	25.33 [a]
		(25.56)	(29.67)	(28.75)	(30.19)

* Mean of three replications.
Means followed by a common letter are not significantly different at 5% level by DMRT
Figures in parentheses are arc sine transformed values.

Effect of calcium chloride on the detection of S.oryzae:

In general, soaking the seeds in different concentrations of $CaCl_2$ has increased *S. oryzae* seed infection count in all the rice seed samples tested (Table 3). Among the different concentrations of $CaCl_2$ tested, soaking the seeds in 2 and 3 per cent concentration recorded more *S.oryzae* seed infection count than other concentrations tested. The maximum *S. oryzae* seed infection count was observed in the variety ADTRH 1 (39.67%) at 2 per cent $CaCl_2$ which followed by ADTRH 1 and CORH 2 seeds soaked at 3 per cent $CaCl_2$ (37.0%). Soaking of seeds in 2 - 3 per cent $CaCl_2$ found better in

Table 3 : Effect of different concentrations of Calcium chloride on the detection of *S.oryzae*

Sr. No.	Concentration	*S. oryzae* infected seeds* (%)					
		IWP	ASD16	ADT36	IR50	ADTRH1	CORH2
1.	1%	22.67[a]	10.33[ab]	28.67[a]	9.33[ab]	30.33[ab]	35.00[b]
		(28.19)	(18.62)	(32.34)	(17.64)	(33.39)	(36.26)
2.	2%	21.33[a]	12.33[ab]	35.00[a]	13.67[b]	39.67[c]	36.00[b]
		(27.47)	(20.50)	(36.18)	(21.59)	(39.03)	(36.82)
3.	3%	23.67[a]	14.67[b]	34.00[a]	12.33[ab]	37.00[bc]	37.00[b]
		(29.04)	(22.39)	(35.63)	(20.55)	(37.46)	(37.46)
4.	5%	19.67[a]	9.00[ab]	29.00[a]	10.33[ab]	33.00[bc]	33.33[b]
		(26.30)	(17.44)	(32.52)	(18.58)	(35.03)	(35.23)
5.	8%	16.33[a]	7.33[a]	25.00[a]	7.00[a]	23.00[a]	21.33[a]
		(23.76)	(15.42)	(29.96)	(15.18)	(28.53)	(27.39)
6.	Blotter (control)	12.00 [b]	10.33 [a]	24.67 [ab]	6.67 [bc]	29.67 [b]	35.67 [ab]
		(19.99)	(18.67)	(29.63)	(14.85)	(32.93)	(36.58)

* Mean of 3 replications; 200 seeds per replication. Figures in parentheses are arc sine transformed values.
Means followed by a common letter are not significantly different at 5% level by DMRT

the seed infection count than standard blotter method. In the present study, soaking the rice seeds in different concentrations of Calcium chloride prior to incubation has improved the detection level of *S.oryzae*. Among the different concentrations of Calcium chloride tested, 2 and 3 per cent were found optimum and recorded maximum *S. oryzae* seed infection count in all the seed sample (Table 3). Renukeswarappa and Sethna (1985) soaked chilli seeds in 5 per cent Calcium chloride solution for 24 hours and plated in Standard blotter method. It yielded 50 per cent more *Colletotrichum dematium*. Rajavel (2000) found increased per cent seed infection count of *Colletotrichum capsici* chilli seeds, when blotters were dipped in 3 per cent Calcium chloride solution before incubation. The method was superior to Standard blotter technique, Deep freeze blotter and 2, 4-D methods. Malone (1962) reported easy detection of *Pyrenophora avenae* (Ito and Kuribay) when oat seeds were exposed to dry heating at 100° C for one hour before plating.

Many detection methods have been developed over the years for various seed-borne pathogens. Several criteria have to be considered in selecting a suitable routine seed health testing procedure for reproducibility. Primarily, it should be capable of revealing maximum pathogen infection and should also be versatile, revealing a range of pathogens (Singh *et al*., 1974). The recent advances in the detection of seed transmitted fungal plant pathogens have come largely through improving existing methods, in particular agar plating and incubation tests. Recent advances in the detection of seed-transmitted fungal plant pathogens have come largely through improving existing methods, in particular agar plating and incubation tests. These methods do not require the use of sophisticated and expensive laboratory equipment, and are thus widely applicable for use in developing countries (Irwin, 1987).

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