

## EFFECT OF SEED-BORNE *SAROCLADIUM ORYZAE*, THE INCITANT OF RICE SHEATH ROT ON RICE SEED QUALITY

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**Abstract:** Rice seeds collected from *Sarocladium oryzae* inoculated plants produced more discoloured grain, chaffiness and recorded much lower seed germination than seeds collected from healthy plants in all the three cultivars tested. The germination, chaffiness and discolouration in healthy plants were found to be in the range of 70.50 to 93.50 per cent, 5.5 to 17.75 per cent and 4 to 18 per cent respectively. There was a progressive and significant reduction in total sugar, reducing sugar and non reducing sugar content of rice seeds with varying degree of seed infection caused by *S. oryzae*. The increase in phenol content due to *S. oryzae* infection ranged from 15.74 to 70.78 per cent and increase being proportional to the per cent *S. oryzae* seed infection.

**Key words:** discolouration, rice seeds, *Sarocladium oryzae*, seed infection, sugars

### INTRODUCTION

Sheath rot caused by *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksw is an important disease of rice, since the pathogen mainly affects the economic part of the rice plant i.e. boot leaf sheath. The fungus is detected frequently during routine seed health testing. The fungus tends to attack leaf sheaths enclosing young panicles, particularly the hybrids, which retards or aborts the emergence of panicles. Seeds from infected panicles become discoloured and sterile, thereby reducing grain yield and quality and become good source of contamination to healthy seeds.

The disease causes empty grain production (Arunyanart *et al.* 1981; Kulwant and Mathur 1992) and glume discolouration (Upadhyay and Diwakar 1984; Vidyasekaran *et al.* 1984; Sachan and Agarwal 1995). It also causes reduction in 1 000 grain weight, poor grain filling and reduction in seed germination (Vidhyasekaran *et al.* 1984). It produces spotting of husks and sterile spike lets (Buddenhagan 1985). Seeds from infected panicles become discoloured and sterile, thereby reducing grain yield and quality (Mew and Gonzales 2002).

Artificially inoculated seeds recorded poor seed germination, root and shoot length reduction among seven rice varieties tested (Lakshmanan 1993). Toxic metabolites of *S. oryzae* caused chlorosis and growth retardation of rice seedlings (Tschen *et al.* 1997; Reddy *et al.* 2000; Ghosh *et al.* 2002; Sakthivel *et al.* 2002).

Pronounced changes in the contents of sugars and starch take place due to fungal infestation in rice grains. Seed protein also showed a marked decrease during the storage of infected rice grains (Bilgrami *et al.* 1979). Sheath rot disease considerably reduced the total sugar content

in all the six varieties tested (Vidyasekaran *et al.* 1984; Reddy *et al.* 2000). Reddy *et al.* (2000) reported that there was a significant decrease in starch content of rice due to *S. oryzae* infection. The present study was carried out to determine the effect of the disease on seed germination, seedling growth and bio-chemical composition of seeds of three important rice cultivars.

### MATERIALS AND METHODS

#### Determination of seed germination, seed discolouration and resulting from chaffiness seed infection *S. oryzae*

Rice seeds of three popular cultivars, CO 43, ASD 18 and ADTRH 1 were used in the study. The seeds were treated with carbendazim @ 2 gm/kg of seeds and stored under laboratory conditions for 24 h. The seeds were then sown in mud pots containing wet land soil in glasshouse and allowed to grow. When the plants were at boot leaf stage, one set of plants were inoculated with *S. oryzae* by single kernel technique (Amin *et al.* 1974). Another set of plants were not inoculated. Seeds were collected from both inoculated as well as healthy plants, shade dried, cleaned and stored under laboratory conditions for further studies.

Germination test was conducted in between paper medium using roll towel technique as described by ISTA (1993). Four replicates of 100 seeds each were randomly counted, placed uniformly in roll towel and kept in the germination room maintained at 25±2°C and 95±3 per cent relative humidity. After 14 days seedlings were evaluated. The germination was expressed as per cent normal seedlings. The per cent discoloured seeds, chaffiness and resultant *S. oryzae* seed infection using standard blotter

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test were recorded. The seed samples were further used for estimating biochemical changes in rice seeds due to *S. oryzae* seed infection.

#### Preparation of ethanol extract

Five grams of seeds were taken from each sample, powdered completely using Willy Mill and plunged into 80 per cent ethanol on boiling water bath for 10 minutes and then cooled in running tap water. The tissues were crushed thoroughly using a pestle and mortar and the macerate was squeezed through two layers of cheese cloth. The extract was collected in a beaker. The residue was again extracted with 80 per cent hot ethanol and squeezed through cheese cloth. Both extracts were pooled together. Then the extract was centrifuged and the supernatant was collected. The supernatant was allowed to evaporate completely. The residue was dissolved in 20 ml of distilled water. This solution was used for further analysis.

#### Estimation of total sugars (Somogyi 1952)

Two ml of solution obtained from ethanol extraction was taken in a test tube and two ml of 5N hydrochloric acid was added. Then this was kept in boiling water bath for 10 minutes with occasional shaking. Later, this was removed from water bath and neutralized with anhydrous sodium carbonate till effervescence ceased. Then the volume was made up to 10 ml using distilled water. The intensity of blue colour was read in Hitachi Spectrophotometer at 520 nm. The amount of total sugar present was estimated.

#### Estimation of reducing sugars (Somogyi 1952)

From the solution obtained after ethanol extraction, the reducing sugar content was estimated.

#### Estimation of non-reducing sugars

The difference between total sugars and reducing sugars yielded the non-reducing sugars.

#### Estimation of starch (Sumner and Somers 1949)

One hundred mg of seeds were taken from each sample, powdered completely using Willy Mill and plunged into 80 per cent ethanol on boiling water bath for 10 minutes and then cooled in running tap water. Ethanol insoluble residue was dried in a hot air oven at 60°C for two days then was placed in a glass stoppered 50 ml Erlenmeyer flask and 12 ml of 6 N HCl was added to the flask and steamed in an autoclave at 110°C for one hour. An aliquot of one ml was withdrawn and the glucose liberated was estimated by the method of Nelson (1944). The amount of starch was determined by multiplying the amount of estimated glucose by 0.9.

#### Estimation of total phenolics (Spies 1955)

Ethanol extract 1 ml was taken in a test tube and the volume was made up to 3 ml with distilled water. Then one ml of Folin's phenol reagent was added to each test tube followed by addition of two ml of 20 per cent sodium carbonate solution. The tubes were kept in boiling water bath for one minute. The test tubes were then cooled and diluted to 10 ml with distilled water. This resulted in deep blue colour solution which was measured at 660 nm. A standard graph was prepared using catechol. The amount of phenol present in the extract was calculated from the standard graph.

## RESULTS

#### Effect of *S. oryzae* on seed germination, seed discoloration and chaffiness

In the present investigation, seeds collected from *S. oryzae* inoculated plants produced more discoloured grain, chaffiness and recorded very low seed germination than the healthy plants in all the three seed samples tested (Table 1). The germination, chaffiness and discoloration in healthy plants were found to be in the range of 70.50 to 93.50 per cent, 5.5 to 17.75 per cent and 4 to 18 per cent, respectively, whereas, the seeds collected from inoculated plants recorded very low seed germination. The chaffi-

Table 1. Effect of *S. oryzae* on seed quality of different rice cultivars

| Serial number. | Variety            | Germination [%]*  | Chaffiness [%]*   | Discolouration [%]* | Resultant seed infection [%]* |
|----------------|--------------------|-------------------|-------------------|---------------------|-------------------------------|
| 1              | Co 43<br>Healthy   | 70.50a<br>(57.35) | 8.75a<br>(16.69)  | 4.00a<br>(11.25)    | 0<br>(0.14)                   |
|                | Inoculated         | 47.75b<br>(43.67) | 40.25b<br>(39.15) | 17.75b<br>(24.45)   | 57.00<br>(62.07)              |
| 2              | ASD 18<br>Healthy  | 93.50a<br>(76.35) | 5.50a<br>(13.39)  | 6.25a<br>(14.20)    | 0<br>(0.14)                   |
|                | Inoculated         | 54.75b<br>(47.83) | 20.25b<br>(26.39) | 11.25b<br>(19.51)   | 42.00<br>(50.62)              |
| 3              | ADTRH 1<br>Healthy | 92.25a<br>(74.18) | 17.75a<br>(24.83) | 18.00a<br>(24.53)   | 0<br>(0.14)                   |
|                | Inoculated         | 49.75b<br>(44.86) | 45.00b<br>(42.09) | 26.25a<br>(30.67)   | 83.00<br>(85.27)              |

\* mean of four replications

Means followed by the same letter are not significantly different at 5% level by DMRT  
Figures in parentheses are arc sine transformed values

ness in seeds collected from inoculated plants ranged from 20.25 to 45.00 per cent. The seed discolouration was also high and ranged from 11.25 to 26.25 per cent.

#### Changes in quality of seeds due to *S. oryzae* infection

When the rice seeds undergo biochemical changes during pathogenesis, the quality of seed is deteriorated. Sugars play an important role in disease development as the pathogens require carbon from sugars for their development and also act as precursors for the synthesis of phenolics, phytoalexins and protein.

In the present study there was a progressive and significant reduction in total sugar, reducing sugar and non-reducing sugar content of rice seeds with varying degree of seed infection caused by *S. oryzae* (Table 2). The same trend was noticed in the case of starch and protein content also. However, there was an increase in total phenol content of infected seeds compared to healthy seeds in all the three seed samples tested. This might be due to the accumulation of phenols by host in response to the invasion by *S. oryzae*.

Table 2. Effect of *S. oryzae* seed infection on total sugar, reducing sugar and non-reducing sugar content of rice seeds

| Serial number | Variety | Seed infection [%]  | Total sugar* [mg/g FW] |                   |                           | Reducing sugar* [mg/g FW] |                    |                           | Non-reducing sugar* [mg/g FW] |                    |                           |
|---------------|---------|---------------------|------------------------|-------------------|---------------------------|---------------------------|--------------------|---------------------------|-------------------------------|--------------------|---------------------------|
|               |         |                     | healthy                | infected          | decrease over healthy [%] | healthy                   | infected           | decrease over healthy [%] | healthy                       | infected           | decrease over healthy [%] |
| 1             | ASD 18  | 42.00<br>(48.22) b  | 5.3 ab<br>(13.258)     | 4.4 b<br>(12.035) | 16.98                     | 2.17<br>(8.45) a          | 1.763<br>(7.624) b | 18.76                     | 2.85<br>(9.72) a              | 2.54<br>(9.17) ab  | 10.88                     |
| 2             | CO 43   | 57.00<br>(66.67) bc | 5.1 a<br>(12.998)      | 3.6 a<br>(10.932) | 29.41                     | 1.97<br>(8.07) a          | 1.170<br>(6.194) a | 40.61                     | 3.18<br>(10.27) ab            | 2.72<br>(9.48) abc | 14.47                     |
| 3             | ADTRH1  | 83.00<br>(84.15) d  | 5.9 c<br>(14.091)      | 3.5 a<br>(10.785) | 40.68                     | 2.83<br>(9.68) b          | 1.290<br>(6.508) a | 54.42                     | 3.28<br>(10.44) b             | 2.36<br>(8.83) a   | 28.05                     |

\* mean of three replications

Means followed by the same letter are not significantly different at 5% level by DMRT  
Figures in parentheses are sine transformed values

## DISCUSSION

The *S. oryzae* infection adversely affected the seed germination, increased chaffiness and discolouration in rice plants. This finding was in agreement with the findings of Reddy *et al.* (2000). The decrease in seed germination and increase in grain discolouration due to sheath rot in different cultivars were also reported by several workers (Pandiaraja-kumar 1992; Mew and Gonzales 2002).

A pronounced decrease in the contents of sugar and starch due to fungal infection of rice grains was reported by several workers. Around 28 per cent reduction in total sugar content of rice seeds due to *S. oryzae* infection in susceptible varieties was recorded whereas it was 17 per cent in resistant varieties. It is therefore evident that *S. oryzae* could utilize well for its growth different sugars present in rice seeds (Bilgrami *et al.* 1979; Reddy *et al.* 2000).

The decrease in starch content, as observed in the present study, might be due to rapid degradation of starch by the enzymes produced by the pathogens or a partial utilization of their synthesis or both (Mirocha and Zaki 1966). Reduction in starch content of rice seeds due to *S. oryzae* infection was reported by Reddy *et al.* (2000).

In the present study, significant changes in protein content were noticed in the seeds with different levels of *S. oryzae* seed infection (Table 3). The seeds of ADTRH 1 with 83.00 per cent *S. oryzae* seed infection recorded 73.46

mg/gm of protein while the healthy seeds of same cultivar recorded 96.57 mg/gm. The decrease in protein content initially might be attributed to their hydrolysis to simpler forms by fungal proteolytic enzymes (Jamaluddin *et al.* 1977). Vidhyasekeran *et al.* (1984) reported that the protein content of healthy rice seeds was 8 per cent, while *S. oryzae* infected seeds recorded 2.2 per cent. Reddy *et al.* (2000) also found the reduction of protein content in the range of 29.7 to 36.7 per cent in rice seeds due to *S. oryzae* infection. However, Sachan and Agarwal (1995) found significant increase in the protein content of seeds having discolouration.

The results of the present study indicated a significant increase in phenolic contents of seeds in all the three cultivars tested. The increase in phenol content due to *S. oryzae* infection ranged from 15.74 to 70.78 per cent and being proportional to per cent *S. oryzae* seed infection. Increase in phenolase activity in the host tissue especially at and around infection sites is a response which characterizes a large number of diseases. A higher level of phenolics may indicate the activation of host defensive system or rapid rate of their synthesis induced by the pathogen leading to tissue necrosis.

Table 3. Effect of *S. oryzae* seed infection on starch, protein and phenol content of rice seed

| Serial number | Variety | Seed infection [%]  | Starch* [mg/g FW]  |                    |                           | Protein* [mg/g FW] |                    |                           | Phenol* [mg/g FW]  |                    |                           |
|---------------|---------|---------------------|--------------------|--------------------|---------------------------|--------------------|--------------------|---------------------------|--------------------|--------------------|---------------------------|
|               |         |                     | healthy            | infected           | decrease over healthy [%] | healthy            | infected           | decrease over healthy [%] | healthy            | infected           | increase over healthy [%] |
| 1             | ASD 18  | 42.00<br>(48.22) b  | 72.66 a<br>(58.49) | 63.79 b<br>(53.01) | 12.21                     | 95.22<br>(77.53) a | 85.92<br>(67.98) c | 9.77                      | 0.209<br>(2.617) a | 0.215<br>(2.656) a | 2.87                      |
| 2             | CO 43   | 57.00<br>(66.67) bc | 74.40 a<br>(59.64) | 63.06 b<br>(52.58) | 15.24                     | 95.51<br>(78.05) a | 81.85<br>(64.80) b | 14.30                     | 0.195<br>(2.528) a | 0.251<br>(2.867) a | 28.72                     |
| 3             | ADTRH1  | 83.00<br>(84.15) d  | 74.57 a<br>(59.78) | 58.02 a<br>(49.62) | 22.19                     | 96.57<br>(79.73) a | 73.46<br>(59.00) a | 23.93                     | 0.219<br>(2.679) a | 0.374<br>(3.502) b | 70.78                     |

\* mean of three replications

Means followed by the same letter are not significantly different at 5% level by DMRT  
Figures in parentheses are sine transformed values

## REFERENCES

- Amin K.S., Sharma B.D., Das C.R. 1974. Occurrence in India – sheath rot of rice caused by *Acrocyliodrium oryzae*. Pl. Dis. Rep. 58: 358–360.
- Arunyanar P., Surin A., Distapon S. 1981. Seed discolouration disease and its chemical control. Int. Rice Res. Newslett. 6 (3), p. 4.
- Bilgrami K.S., Jamaluddin R.K.S., Sinha K., Prasad T. 1979. Changes in seed contents of paddy due to fungal flora. J. Phytopathol. 96: 9–14.
- Buddenhagan I.W. 1985. Rice disease evaluation in Madagascar. Int. Rice Res. Newslett. 1: 74–75.
- Ghosh M.K., Amudha R., Jayachandran S., Sakthivel N. 2002. Detection and quantification of phytotoxic metabolites of *Sarocladium oryzae* in sheath rot infected grains of rice. Lett. Appl. Microbiol. 34 (6): 398–401.
- ISTA 1993. International rules for seed testing. Seed Sci. Technol. 21: 141–186.
- Jamaluddin K.S., Bilgrami K.S., Prasad T. 1977. Changes in protein contents of *Phaseolus mungo* due to fungal flora. Curr. Sci. 46, p. 461.
- Kulwant S., Mathur S.B. 1992. Further evidence of transmission of *Sarocladium oryzae* through rice seeds and its quarantine significance. Ind. Phytopathol. 45: 454–456.
- Lakshmanan P. 1993. Studies on sheath rot disease of rice due to the interactions of *Sarocladium oryzae* and rice insect pests. Ph.D. Thesis, Tamil Nadu Agricultural University, Coimbatore, India, 211 pp.
- Mew T.W., Gonzales 2002. A Handbook of Rice Seed-Borne Fungi. IRRI Science Publishers, 83 pp.
- Mirocha C.J., Zaki A.I. 1966. Fluctuation in amount of starch in host plants invaded by rust and mildew fungi. Phytopathology 56: 1220–1224.
- Nelson N. 1944. A photometric adaptation of somogyi method for the determination of glucose. J. Biol. Chem. 153: 375–380.
- Pandiarajakumar S. 1992. Studies on sheath rot of rice caused by *Sarocladium oryzae*. M.Sc (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, India, 165 pp.
- Reddy M.M., Reddy C.S., Gopal S.B. 2000. Effect of sheath rot disease on quality characters of rice grain. J. Myc. Plant Pathol. 30 (1): 68–72.
- Sachan I.P., Agarwal V.K. 1995. Seed discolouration of rice: location of inoculum and influence on nutritional value. Indian Phytopathol. 48 (1): 14–20.
- Sakthivel N., Amudha R., Muthukrishnan S. 2002. Production of phytotoxic metabolites by *Sarocladium oryzae*. Mycol. Res. 106 (5): 609–614.
- Somogyi M. 1952. Notes on sugar determination. J. Biol. Chem. 200: 145–154.
- Spies J.R. 1955. Estimation of phenols. p. 467–468. In: "Methods in Enzymology" (3rd ed.) (S.P. Clockwick, W.O. Kaplan, eds.). Academic Press, London, 748 pp.
- Sumner J.B., Somers G.F. 1949. Laboratory Experiments in Biological Chemistry. Academic Press, New York, 167 pp.
- Tschen Li-Ling Ch., Shean-Tzong H., Tian-Shung Wu. 1997. Isolation and phytotoxic effects of helvolic acid from plant pathogenic fungus *Sarocladium oryzae*. Bot. Bull. Acad. Sin. 38: 251–256.
- Upadhyay R.K., Diwakar M.C. 1984. Sheath rot in Chatisgarh, Madya Pradesh, India. Int. Rice Res. Newslett. 9 (5), p. 5.
- Vidhyasekaran P., Ranganathan K., Rajamanickam 1984. Quality of rice grains from sheath rot affected plant. Int. Rice Res. Newslett. 12 (1), p. 17.

**POLISH SUMMARY****WPŁYW PRZENOSZĄCEGO SIĘ PRZEZ  
NASIONA PATOGENA *SAROCLADIUM ORYZAE*,  
CZYNNIKA SPRAWCZEGO GNICIA POCHEW  
RYŻU, NA JAKOŚĆ NASION**

Nasiona ryżu trzech badanych odmian zebrane z roślin inokulowanych grzybem *Sarocladium oryzae* były bardziej przebarwione, wytwarzały więcej plew i znacznie

gorzej kiełkowały niż nasiona roślin zdrowych. Kiełkowanie, oplewienie i przebarwienie zdrowych roślin mieściło się, odpowiednio, w granicach od 70,50 do 93,50%, od 5,5 do 17,75% i od 4 do 18%. W różnym stopniu występowała znaczna redukcja zawartości cukrów ogółem, cukrów redukujących i nieredukujących w porażonych nasionach ryżu. Wzrost zawartości fenoli wywołany porażeniem przez *S. oryzae* wahał się w granicach od 15,74 do 70,78% i był proporcjonalny do procentu porażenia nasion przez *S. oryzae*.