Location and seed transmission of fungi in discoloured seeds of hybrid rice

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ABSTRACT: The seed-borne inoculum of Alternaria padwickii, Cochliobolus miyabeanus, Drechslera australiensis, Fusarium pallidoroseum and Magnaporthe grisea was detected only from seed coat and endosperm. Whereas Cochliobolus lunatus, Curvularia ovoidea, C. oryzae, Gibbrella fujikuroi, Magnaporthe salavinii, Monographella albescens, Nigrospora oryzae and Sarocladium oryzae were detected from all parts of seed i.e. seedcoat, endosperm and embryo. Seed infection with M. salvinii resulted in seedling rot and seedling mortality. The maximum seedling mortality was found in seeds with discolouration on both endosperm plus embryo regions.

Key words : Hybrid rice, seed-borne fungi, seed transmission

Great strides have been made during last two decades in development of high yielding hybrids of rice throughout the world. To attain high yield level, it is necessary that the hybrids released should be relatively resistant to plant diseases, majority of which are seedborne in nature (Ou, 1985; Mew et al., 1988; Agarwal et al., 1989; Singh, 1993). Rice is mostly grown in the wet season, when high humidity and high temperature prevail. These conditions are congenial for infection of seed with number of fungi. There are possibilities of higher seed infection in hybrids as compared to conventional varieties because of the parental lines having open flower type and exposure of flowers for a longer period of time. There is very little information available on seedborne fungi and seed discoloration in hybrid rice. Keeping this in view, the present studies were conducted to determine the location of seedborne fungi and their seed transmission in different hybrids of rice.

MATERIALS AND METHODS

Freshly harvested seeds of hybrid rice were obtained from Project Coordinator, Hybrid Rice, G.B. Pant University of Agriculture & Technology, Pantnagar, Maharashtra Hybrid Seed Corporation (MAHYCO) Mumbai and National Seed Corporation (NSC) Delhi. The seeds exhibiting ten types of discolorations, namely, ash gray, ash gray with black dots, compact orange, dark brown and fluffy black (in Pant Sankar Dhan-I), black dots (in PRH-1), eye shaped spots (in UPHR-832), light brown, light to dark brown spots (in MPH-529), pale yellow discoloration (in APHR-2) on embryo region, 1-25%, 26-50%, 51-75%, 76-100% endosperm region and on both embryo plus endosperm region were selected for the present studies.

Location of inoculum in seed was determined by seed component plating (Mathur et al., 1975). Surface sterilized seeds were soaked individually in sterilized water for 15 h at 25°C. Each seed was then dissected aseptically in different parts i.e. seed coat, endosperm and embryo with the help of sterilized forceps and knife. Different parts of one seed were plated on PDA in one plate and incubated at 25°C. Fungi isolated from each part of the seed were recorded and identified. Transmission of fungi from seed to seedling was determined by following methods:

Seedling symptomatology test in agar test tubes

Surface sterilized seeds were placed in test tube containing 15 ml of 1% water agar medium, one seed per tube with the help of sterilized forceps aseptically (Khare et al., 1977). One hundred seeds of each category of discoloration were tested. These test tubes were incubated for 14 days at 25°C. After removal of cotton plug on 4th day and observed for seedling blight, seed rot and coleoptile spots. Subsequently isolations of fungi were made from seed rot and seedlings exhibit-
Sterilized sand method

In this method, sterilized sand was filled in pots of 250 g capacity and surface sterilized seeds were sown one seed per pot (Agarwal and Sinclair, 1996). Observations were recorded after 15 days on abnormal seedlings, seed rot and coleoptile spots in the glasshouse. Later, isolations of fungi were made from diseased seed and seedlings.

RESULTS AND DISCUSSION

Location of inoculum

The results revealed that, in general, per cent incidence of fungi in different parts of seed varied with regard to degree of seed discolouration. A higher incidence of infection was detected in seed parts from seeds with higher degree of discolouration. Information pertaining to the incidence of fungi in all the ten types of discolourations with various degrees of discolouration is as follows:

**Alternaria padwickii**

The infection of *A. padwickii* was detected from seed coat and endosperm in seeds exhibiting ash gray, ash gray with black dots, black dots, dark brown, light brown, light to dark brown spots and pale yellow discolourations. The average per cent incidence of *A. padwickii* in seeds showing ash gray, ash gray with black dots, black dots, dark brown, light brown, light to dark brown spots and pale yellow discolourations was 30.0, 32.5, 26.6, 24.0, 14.0, 12.0, 30.0 per cent in seed coat and 20.0, 27.5, 20.0, 14.0, 10.0, 4.0, 12.0 per cent in endosperm, respectively.

**Cochliobolus lunatus**

The infection of *C. lunatus* was detected from all parts of seed, i.e., seed coat, endosperm and embryo in discolourations, namely, ash gray, ash gray with black dots, black dots, fluffy black, compact orange, dark brown and light brown discolourations and from seed coat and endosperm in light to dark brown spots and pale yellow discolourations. The average per cent incidence of *C. lunatus* in seeds with ash gray, ash gray with black dots, black dots, fluffy black, compact orange, dark brown, light brown discolourations was 20.0, 25.0, 36.6, 96.6, 20.0, 28.0, 16.0 per cent in seed coat, 22.0, 22.5, 33.3, 100.0, 16.0, 20.0, 8.0 per cent in endosperm and 8.0, 7.5, 10.0, 66.6, 2.5, 6.0, 6.0 per cent in embryo, respectively. Whereas the average per cent incidence of *C. lunatus* in seeds with light to dark brown spots, eye shaped spots and pale yellow discolourations was 22.0, 20.0, 22.0 per cent in seed coat and 6.0, 10.0, 4.0 in endosperm region, respectively.

**Cochliobolus miyabeanus**

The infection of *C. miyabeanus* was found in seed coat, endosperm and embryo depending on the type of discolouration. Infection of *C. miyabeanus* was detected in seed coat and endosperm in seeds exhibiting black dots, eye shaped spots, light to dark brown spots and pale yellow discolourations in varying proportion. The average per cent incidence of *C. miyabeanus* from seeds exhibiting black dot and pale yellow discolourations was 16.6, 14.0 and 10.0, 4.0 per cent in seed coat and endosperm, respectively. In seeds with light to dark brown spots the incidence of *C. miyabeanus* was 22.0, 20.0 and 6.0 per cent in seed coat, endosperm and embryo, respectively. The fungus was isolated only from seed coat of seeds exhibiting eye shaped spots at an average incidence of 10.0 per cent.

**Curvularia oryzae**

The infection of *C. oryzae* was detected from all parts of seeds showing light to dark brown spots. The

### Table 1. Assessment of seed transmission of seedborne fungi in different discolourations in hybrid rice

<table>
<thead>
<tr>
<th>Types of discolouration</th>
<th><em>A. padwickii</em></th>
<th><em>C. lunatus</em></th>
<th><em>C. miyabeanus</em></th>
<th><em>G. fujiiroii</em></th>
<th><em>M. salvinii</em></th>
<th><em>M. albuscens</em></th>
<th><em>N. oryzae</em></th>
<th><em>S. oryzae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>14</td>
<td>0</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Ash Gray</td>
<td>38</td>
<td>16</td>
<td>0</td>
<td>2</td>
<td>36</td>
<td>0</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Black dots</td>
<td>6</td>
<td>22</td>
<td>8</td>
<td>4</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dark brown</td>
<td>12</td>
<td>12</td>
<td>0</td>
<td>6</td>
<td>38</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Light brown</td>
<td>4</td>
<td>26</td>
<td>2</td>
<td>4</td>
<td>52</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Light to dark</td>
<td>18</td>
<td>12</td>
<td>26</td>
<td>0</td>
<td>20</td>
<td>2</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Brown spots</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pale yellow</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>16</td>
<td>64</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

1. Data are based on observation of 100 seeds.
2. Per cent transmission of fungi is based on isolation from seedlings, showing symptoms/seed rot.
3. Seedling exhibiting symptoms are recorded up to 15 days after germination.
average per cent incidence was 24.0, 20.0 and 6.0 in seed coat, endosperm and embryo, respectively.

**Curvularia ovoidea**

The infection of *C. ovoidea* was detected from seed coat and endosperm of seeds exhibiting ash gray discoulouration and ash gray with black dots and all parts of seed having dark brown discoulouration. The average per cent incidence of *C. ovoidea* in seeds with ash gray discoulouration and ash gray with black dots was 10.0, 15.0 in seed coat and 6.0, 7.5 per cent in endosperm, respectively. Whereas, in seeds showing dark brown discoulouration, the average per cent incidence was 16.0, 6.0 and 2.0 in seed coat, endosperm and embryo respectively.

**Drechslera australiensis**

The infection of *D. australiensis* was detected from seed coat and endosperm of seeds exhibiting ash gray, ash gray with black dots and dark brown discoulourations and from seed coat of seeds exhibiting fluffy black discoulouration. The average per cent incidence of *D. australiensis* in seeds showing ash gray, ash gray with black dots, and dark brown discoulourations was 6.0, 32.0, 30.0 and 4.0, 25.0,24.0 per cent in seed coat and endosperm, respectively. Whereas, in fluffy black discoulouration, *D. australiensis* was detected only from seed coat.

**Fusarium pallidoroseum**

Infection of *F. pallidoroseum* was detected from the seeds exhibiting dark brown discoulouration in seed coat and endosperm. The average per cent incidence of *F. pallidoroseum* was 16.0 and 12.0 per cent from seed coat and endosperm, respectively.

**Gibberella fujikuroi**

The infection of *G. fujikuroi* was detected from all parts of seed, i.e., seed coat, endosperm and embryo. The average per cent incidence of *G. fujikuroi* in seeds exhibiting ash gray discoulouration, ash gray with black dots and light brown discoulouration was 14.0, 17.5, 20.0 per cent in seed coat and 10.0, 5.0, 6.0 per cent in endosperm, respectively. Whereas in seeds exhibiting black dots, compact orange, dark brown discoulouration and light to dark brown spots the average per cent incidence of *G. fujikuroi* was 20.0, 74.0, 14.0, 24.0 per cent in seed coat and 16.6, 100.0, 8.0, 10.0 per cent in endosperm and 3.3, 58.0, 2.0, 6.0 in embryo, respectively. From the seed coat of seeds exhibiting fluffy black symptoms, *G. fujikuroi* was detected at an average incidence of 16.6 per cent. In addition, *G. fujikuroi* was also detected from seed coat of healthy seeds of hybrids Pant Sankar Dhan-I and PRH-1 at lower incidence.

**Magnaporthe grisea**

The infection of *M. grisea* was detected only from seed coat and endosperm of seeds exhibiting eye shaped spots. The average per cent incidence of *M. grisea* was 70.0 and 80.0 per cent from seed coat and endosperm, respectively.

**Magnaporthe salvinii**

Infection of *M. salvinii* was detected from seeds exhibiting ash gray discoulouration ash gray with black dots, black dots, dark brown, light brown, light to dark brown spots and pale yellow discoulourations. In addition, *M. salvinii* was also detected from seed coat and endosperm of healthy seeds. In seeds exhibiting ash gray, ash gray with black dots, black dots, dark brown, light brown, light to dark brown spots and pale yellow discoulouration, the average per cent incidence of *M. salvinii* was 36.0, 27.5, 36.6, 40.0, 48.0, 30.0, 16.0 per cent in seed coat and 32.0, 25.0, 36.6, 38.0, 36.0, 16.0 and 20.0 per cent in endosperm and 8.0, 7.5, 13.3, 10.0, 18.0, 8.0, 6.0 per cent in embryo, respectively.

**Monographella albescens**

This fungus was detected from all parts of seed exhibiting pale yellow discoulouration. The average per cent incidence was 54.0, 32.0 and 16.0 from seed coat, endosperm and embryo respectively.

**Nigrospora oryzae**

This fungus was detected from all parts of seeds showing ash gray discoulouration and ash gray with black dots. The average per cent incidence was 24.0, 16.0 and 6.0 from seed coat, endosperm and embryo in seeds showing ash gray discoulouration. In case of seeds showing ash gray discoulouration with black dots average per cent incidence of *N. oryzae* was 22.5, 12.5 and 5.0 from seed coat, endosperm and embryo, respectively.

The location of seed-borne inoculum was found to vary depending upon the pathogen and degree of seed infection. In all the cases, per cent recovery of fungi from different parts of hybrid rice seed varied depending on degree of discoulourations. A higher per cent recovery of the pathogens was found when both embryo plus endosperm regions were discouloured as compared to lower degree of discoulourations. Similarly,
Sachan and Agarwal (1995) also reported a higher percentage of occurrence of pathogens in different parts of seeds of conventional rice varieties when the degree of discolouration was higher.

Earlier reports indicate the infection of *G. zeae*, *N. oryzae* and *G. fujikuroi* in seed coat, endosperm and embryo of conventional rice seeds (Mathur et al., 1975; Agarwal et al., 1989; Sachan and Agarwal 1995). Zakeri and Zad (1983), Sachan and Agarwal (1995) reported *C. lunatus* and *C. geniculatus* from seed coat, endosperm and embryo of conventional rice seeds. Likewise, *M. albescens* was also detected from seed coat, endosperm and embryo regions of conventional rice seeds (Mia et al., 1986). Chaung and Lee (1983) reported infection of *M. grisea* in all parts of conventional rice seeds. Sachan and Agarwal (1995) reported *C. miyabeanus* from seed coat and endosperm in conventional rice seeds.

**Seed transmission**

Transmission of seedborne fungi in seeds exhibiting ash gray discolouration, black dots, dark brown, light brown, light to dark brown spots and pale yellow discolourations revealed an overall higher percentage seed transmission of *M. salvinii* in all types of discolourations. Seed transmission of *M. salvinii* was 36.0, 18.0, 38.0, 20.0, 16.0 and 14.0 per cent in seeds exhibiting ash gray, black dots, dark brown, light brown, light to dark brown spots, pale yellow discolourations and healthy seeds, respectively. Seed transmission of *A. padwickii* was 38.0, 6.0, 12.0, 4.0, 18.0, 2.0 and 4.0 per cent in ash gray, black dots, dark brown, light brown, light to dark brown spots, pale yellow discolourations and healthy seeds, respectively. Seed transmission of *N. oryzae* was 18.0 and 2.0 per cent in seeds exhibiting black dots and dark brown discolourations, respectively Seed transmission of *Sarocladium oryzae* was 6.0, 16.0 and 2.0 per cent in ash gray discolouration, light to dark brown spots and pale yellow discolouration respectively (Table 1).

Seed transmission of *M. salvinii* from heavily infected seeds of hybrid rice MPH-529 exhibiting light brown discolouration was studied in detail by agar test tube method and sterilized sand method in glasshouse conditions. The seeds having all degrees of discolourations were used for assessment of per cent seed transmission of *M. salvinii* and observations were recorded on germination, seedling infection and seedling mortality. The reduction in germination increased, with increase in degree of discolouration. The germination was 68.0 and 61.0 per cent in agar test tube and sterilized sand method, respectively, in case of seeds exhibiting yellow discolourations and healthy seeds, respectively. Seed transmission of *C. miyabeanus* was 8.0, 2.0, 26.0, 8.0 and 2.0 per cent in seeds with black dots, light brown, light to dark brown spots, pale yellow discolourations and healthy seeds, respectively. In seeds exhibiting ash gray, black dots, dark brown, light brown, light to dark brown spots, pale yellow discolourations and healthy seeds, the per cent seed transmission of *C. lunatus* was 16.0, 12.0, 26.0, 12.0, 4.0 and 4.0 per cent, respectively. Percent seed transmission of *G. fujikuroi* was 2.0, 4.0, 6.0, 4.0, 6.0 and 2.0 in ash gray, black dots, dark brown, light brown, pale yellow discolourations and healthy seeds, respectively. Transmission of *M. albescens* in seeds with light brown, light to dark brown spots and pale yellow discolourations was 2.0, 2.0 and 64.0 per cent, respectively. Seed transmission of *N. oryzae* was 18.0 and 2.0 per cent in seeds exhibiting black dots and dark brown discolourations, respectively Seed transmission of *Sarocladium oryzae* was 6.0, 16.0 and 2.0 per cent in ash gray discolouration, light to dark brown spots and pale yellow discolouration respectively (Table 1).

### Table 2. Seed transmission of *M. salvinii* in dicoloured seeds of hybrid rice MPH-529

<table>
<thead>
<tr>
<th>Degree of discolouration</th>
<th>Agar test tube method</th>
<th>Sterilized sand method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Germination (%)</td>
<td>Healthy seedlings (%)</td>
</tr>
<tr>
<td>A. Healthy</td>
<td>98</td>
<td>76</td>
</tr>
<tr>
<td>B. Discoloured</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a. Only endosperm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>i. 1-25% surface area</td>
<td>87</td>
<td>58</td>
</tr>
<tr>
<td>ii. 26-50% surface area</td>
<td>82</td>
<td>50</td>
</tr>
<tr>
<td>iii. 51-75% surface area</td>
<td>73</td>
<td>37</td>
</tr>
<tr>
<td>iv. 76-100% surface area</td>
<td>71</td>
<td>23</td>
</tr>
<tr>
<td>b. Embryo and</td>
<td>68</td>
<td>17</td>
</tr>
<tr>
<td>Endosperm both</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Data based on observation of 100 seeds in each case.
2. Per cent transmission of fungi determined by isolation from seedlings showing symptoms/seed rot.
3. Seedling exhibiting symptoms recorded up to 15 days after germination.
having endosperm plus embryo both discoloured. Whereas, in apparently healthy seeds, germination was 98.0 and 86.0 per cent in agar test tube and sterilized sand method, respectively. Ten days after germination, the seedlings in both agar test tubes and sterilized sand developed typical stem rot symptoms. A total of 51.0 and 49.0 per cent seedlings developed symptoms in case of seeds exhibiting discolouration in endosperm plus embryo, in agar test tube and sterilized sand, respectively. Fifteen days after germination, seedling mortality was 49.0 and 46.0 per cent in agar test tube method and sterilized sand method, respectively (Table 2). Isolations made from symptoms and seedling mortality yielded \textit{M. salvinii} in all cases.

Earlier Ahmed and Prasad (1985), Ou (1985), Agarwal \textit{et al.}, (1989) found seedling mortality, root browning, collar, necrosis and coleoptile spots due to \textit{C. miyabeae} infection conventional rice. Earlier Milagrosa (1987) reported that \textit{S. oryzae} does not produce any visible symptoms on young seedlings. Narain (1992) assumed that seed rot and seedling mortality due to \textit{S. oryzae} infection may be the result of toxin production.

A maximum transmission of \textit{M. salvinii} (49.0 - 51.0\%) from seeds to seedlings was observed in seeds exhibiting light brown discolouration in rice hybrid MPH 529. The infected seed resulted in seedlings with typical stem rot symptoms on seedlings and the outer leaf sheath rotted causing mortality of seedlings. In later stages, small black dot like sclerotia were also observed on infected region. Ou (1985) considered \textit{M. salvinii} primarily as wound pathogen in conventional rice. However, in case of hybrid rice, it has been included as a major pathogen (Anonymous, 1996). In hybrid rice it was detected from all parts of seed. This clearly indicates its internally seedborne nature in hybrid rice. The open glumes with longer duration of exposure of stigma of CMS lines (seed parent) together with increased doses of N and P fertilizers in hybrid rice seed production are the major factors for higher infection of \textit{M. salvinii} in hybrid rice seeds. Therefore, the per cent incidence of \textit{M. salvinii} was found higher in hybrid rice and may become a major seed-borne pathogen in hybrid rice in years to come.

REFERENCES


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