Rice blast-mycoflora, symptomatology and pathogenicity

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Abstract

Rice (Oryza sativa L.) is the second most important cereal crop of the world as well as of Pakistan. Rice blast caused by a Magnaporthe oryzae (syn: Pyricularia oryzae Cav.) is an infectious fungal disease which is distributed worldwide and prevailing in more than 85 countries of the world. Therefore, present studies were carried out to isolated different fungi associated with seeds and leaves of commercially grown rice varieties and to test the pathogenicity of predominantly isolated species. Seven fungi namely Magnaporthe oryzae, Curvularia lunata, Helminthosporium oryzae, Fusarium moniliforme, Alternaria alternata, Nigrospora oryzae and Aspergillus niger were isolated from seeds and affected leaves of five rice varieties viz., IRRI-6, IRRI-8, DR-82, KS-282 and Shua. N. oryzae was isolated only from IRRI-6 and IRRI-8 rice varieties. Among these fungi, M. oryzae was predominantly isolated both from seeds and leaves of the rice varieties studied. Rice variety IRRI-6 followed by KS-282 appeared as most susceptible rice cultivars to rice blast fungus. M. oryzae was detected with highest frequency from leaves and seeds of IRRI-6. All other fungi were isolated either occasionally or with low frequency. Pathogenicity test of M. oryzae conducted on apparently most susceptible variety IRRI-6 has confirmed the pathogenic nature of the fungus. Plant growth was significantly decreased in inoculated plants as compared to un-inoculated plants. Moreover, typical rice blast disease symptoms were also produced in inoculated plants.

Key words: Rice blast, fungi, Magnaporthe oryzae, symptomatology, pathogenicity

Introduction

Rice (Oryza sativa L.) is the second most important cereal crop of the world. It is staple food for more than half of the world’s population. Approximately 90% of global rice production is contributed by Asia continent alone¹⁵. Rice is also important staple food and cash crop of Pakistan. Rice crop is subjected to attack of 50 diseases that including 6 bacterial, 21 fungal, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders¹²,³¹. However, major diseases are rice blast, brown spot, bacterial leaf blight and leaf streak, sheath blight, sheath rot, Fusarium wilt or Bakanae, stem rot, Tungro virus, false smut and post-harvest diseases²³. These diseases either attack at any growth stage of rice plant or infect rice grains after harvest, causing considerable losses in both quality and quantity of the produce. It is estimated that about 14-18% yield reduction was caused by these diseases worldwide¹⁷.
Rice blast caused by a filamentous, ascomycete fungus *Magnaporthe oryzae* (syn: *Pyricularia oryzae* Cav.) is an infectious fungal disease which is distributed worldwide and prevailing in more than 85 countries of the world\(^9\,22\). Blast disease occurs in a wide range of climatic conditions from temperate to tropics and the pathogen is spread by wind and disseminated by infected plant debris or seeds left in the fields\(^3\). Rice blast can be appeared at any stage of growth and produced various symptoms\(^13\). Several rice blast epidemics have occurred in different parts of the world, resulting in yield losses in these areas ranging from 50 to 90% of the expected crop\(^1\). Under usual conditions yield losses due to blast ranged from 1-50% in different rice growing regions of the world depending upon the type of cultivars grown and environmental conditions prevailed\(^10\). However, under favourable environmental conditions the disease caused heavy losses and yield may reach up to 90%\(^16\). In south and south east Asia the losses due to blast was estimated about US$55 million annually\(^21\). Rice blast is also recognized as one of the important disease in Pakistan causing considerable losses in yield\(^11\,24\). Studies were, therefore, carried out to study the symptomatology and isolation of fungi associated seeds and leaves of commonly growing rice varieties. Pathogenicity of the most frequently isolated fungal pathogen was also tested which is presented herein.

**Materials and methods**

**Collection of samples:** Diseased samples consisting of seeds and leaves of IRRI-6, IRRI-8, KS-282, DR-82 and Shua rice varieties, were collected from different locations of district Badin. These rice varieties are commonly cultivated in district Badin. These samples were placed in paper bags, which were properly labeled and brought to the laboratory for isolation of disease causing fungi.

**Isolation and purification of fungi:** For isolation, affected seeds and leaves were surface sterilized with 5% commercial bleach (Sodium hypochlorite) for 1-1.5 minutes and then placed in sterilized Petri dishes containing freshly prepared potato dextrose agar (PDA) medium. Five seeds/pieces of diseased plant parts were placed in each Petri dish. These Petri dishes were incubated at 25°C for five days to induce sporulation of the fungi. Different fungal colonies were appeared, which were purified and multiplied on PDA. The isolated fungal species were identified on the basis of their morphological characteristics with the help of keys by Barnett and Hunter\(^3\), Booth\(^5\), Domsch *et al.*\(^7\), Ellis\(^8\), Singh\(^26\) and Sutton\(^28\). The data on frequency of isolated fungi from seeds and leaves of different rice varieties were recorded using the following formula:

\[
\text{Colonization} \% = \frac{\text{Number of seeds/pieces colonized by a fungus}}{\text{Total number of seeds/pieces studied}} \times 100
\]

**Pathogenicity test:** Pathogenicity test of most frequently isolated fungus, *Magnaporthe oryzae* Couch was carried out to confirm the etiology of the disease. For this purpose nursery of IRRI-6 was raised in earthen pots thoroughly washed with spirit containing 2 kg sterilized
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soil. After 20 days nursery was transplanted in earthen pots containing sterilized soil at one plant per pot.

**Preparation of conidial suspension:** For inoculation, conidial suspension was prepared from 14 days old culture of *Magnaporthe oryzae*. 10 ml of sterilized water was added in PDA plates containing culture of the test fungus. To remove the conidia, the culture was rubbed with sterilized hair brush gently and the spore suspension was collected in a sterilized glass beaker. The suspension was adjusted to $10^5$ conidia per milliliter of water\(^6\). This was done with the help of hemocytometer\(^3\).

**Inoculation of pathogen inoculums:** The prepared conidial suspension of the fungus was inoculated at fourth leaf stage of rice plant by spraying approximately at 0.2 ml/plant\(^4\). The inoculated plants were incubated at 25 to 28°C under 100% humidity for 72 hours under darkness for penetration of conidia and disease development. The humidity was maintained by using humidifier and then was exposed to open air. The un-inoculated plants were served as control. There were 15 replications (each earthen pot containing one plant) of both inoculated and un-inoculated. After 15 days of inoculation, data on disease incidence, severity, plant height and weight was recorded.

**Re-isolation of the fungus:** Re-isolation was also done from inoculated and un-inoculated plants as described above to confirm the pathogenic nature of the test fungus. For this purpose, leaves were washed thoroughly with tap water and cut into small pieces. After surface sterilization with 5% bleach solution, pieces were placed on PDA plates. The recovery of inoculated fungus was recorded and infection percent was calculated with the help of following formula.

\[
\text{Infection \%} = \frac{\text{Number of pieces colonized by the fungus}}{\text{Total number of pieces studied}} \times 100
\]

While disease incidence and severity were calculated with following formulas;

\[
\text{Disease incidence \%} = \frac{\text{Number of infected plants with the disease}}{\text{Total number of plants studied}} \times 100
\]

\[
\text{Disease severity \%} = \frac{\text{Area of plant covered by the disease}}{\text{Total area of the plant studied}} \times 100
\]

Finally the data was analyzed by ANOVA using Statistix 8.1 software. Least significant differences (LSD) were calculated using significant level at $P = 0.05$.

**Results**

**Disease symptoms:** The rice blast fungus caused infection on all growth stages of rice plant. On leaves of susceptible rice variety the disease initially appeared as whitish or grayish specks along
the leaf margins (Fig. 1a). Later on they turned into elliptical spots which are elongated and diamond shaped with pointed ends (Fig. 1b). These spots became necrotic in the center with brown or reddish-brown margins (Fig. 1c). These spots collapse each other and forms large lesions (Fig. 1c). On stem, fungus produced elongated, grayish to black colour lesions (Fig. 1d). Disease also appeared on rice seeds as brown diamond shaped spot (Fig. 1e).

Table- 1. Frequency of isolated fungi associated with leaves of different rice varieties.

<table>
<thead>
<tr>
<th>Isolated fungi</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IRRI-6</td>
</tr>
<tr>
<td><strong>Magnaporthe oryzae</strong></td>
<td>52</td>
</tr>
<tr>
<td><strong>Curvularia lunata</strong></td>
<td>16</td>
</tr>
<tr>
<td><strong>Helminthosporium oryzae</strong></td>
<td>10</td>
</tr>
<tr>
<td><strong>Fusarium moniliforme</strong></td>
<td>8</td>
</tr>
<tr>
<td><strong>Alternaria alternata</strong></td>
<td>6</td>
</tr>
<tr>
<td><strong>Nigrospora oryzae</strong></td>
<td>-</td>
</tr>
<tr>
<td><strong>Aspergillus niger</strong></td>
<td>8</td>
</tr>
</tbody>
</table>

**Isolation of fungi from leaves of different rice varieties:** Seven fungi namely *Magnaporthe oryzae, Curvularia lunata, Helminthosporium oryzae, Fusarium moniliforme, Alternaria alternata, Aspergillus niger* and *Nigrospora oryzae* were isolated from the leaves of five different rice varieties (Table 1). *N. oryzae* was isolated only from IRRI-8 rice variety (Table 1). The association of different fungi is greatly varied with rice cultivars. Among the seven fungi, the frequency of rice blast fungus, *M. oryzae* was significantly high and as compared to the other fungi. *M. oryzae* appeared as the pre-dominant fungus associated (Table 1). The maximum infection of *M. oryzae* is recorded in variety IRRI-6 (52%) followed by KS-282 (50%) and IRRI-8 (46%). Whereas, minimum infection of *M. oryzae* was found on variety Shua (36%) followed by DR-82 (40%). As compared to *M. oryzae*, all other fungi were isolated in low frequencies from the infected leaves of rice varieties (Fig. 2).
In aggregate, the *M. oryzae* also appeared to be the pre-dominant fungus, which isolated in very high frequency (44.8%) from leaves of the all the five varieties (Fig. 2). The *C. lunata* stood second (17.2%) followed by *H. oryzae* (12%). All other fungi were rarely isolated from infected leaves of rice varieties.

<table>
<thead>
<tr>
<th>Isolated fungi</th>
<th>IRRI-6</th>
<th>IRRI-8</th>
<th>DR-82</th>
<th>KS-282</th>
<th>SHUA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Magnaporthe oryzae</em></td>
<td>23</td>
<td>20</td>
<td>21</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td><em>Curvularia lunata</em></td>
<td>17.5</td>
<td>19</td>
<td>18</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td><em>Helminthosporium oryzae</em></td>
<td>15</td>
<td>17</td>
<td>19</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td><em>Fusarium moniliforme</em></td>
<td>12</td>
<td>14</td>
<td>11</td>
<td>13</td>
<td>8</td>
</tr>
<tr>
<td><em>Alternaria alternata</em></td>
<td>14</td>
<td>10</td>
<td>8</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td><em>Nigrospora oryzae</em></td>
<td>-</td>
<td>8</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>13.5</td>
<td>11</td>
<td>6</td>
<td>16</td>
<td>12</td>
</tr>
</tbody>
</table>

**Isolation of fungi from seeds of different rice varieties:** Six fungi viz., *C. lunata*, *H. oryzae*, *F. moniliforme*, *A. alternata*, *N. oryzae* and *A. niger* were isolated from seeds of five rice varieties namely IRRI-6, IRRI-8, DR-82, KS-282 and Shua (Fig. 3). The rice blast pathogenic fungus, *M. oryzae* followed by *C. lunata* were the predominant fungi associated with seeds of all five varieties with high frequencies. *N. oryzae* was isolated from seeds in very low frequencies of only two rice varieties i.e. IRRI-8 and DR-82 (Table 2).

The frequency of *M. oryzae* in all varieties was ranging from 19-23% with highest infection on IRRI-6 (23%) followed by DR-82 (21%). Whereas the frequency of *C. lunata* from seeds of rice varieties was ranging from15.0-19.0% (Table 2). However, *N. oryzae* was isolated from very few seeds of DR-82 (4.0%) and IRRI-8 (8.0%) respectively, (Table 2).

**Pathogenicity test of Magnaporthe oryzae:** *M. oryzae* influenced significantly on the growth of rice plants and produced typical rice blast disease symptoms on inoculated plants of IRRI-6
variety. Plant height and weight were significantly reduced in plants inoculated with the fungus as compared to un-inoculated plants (Fig. 4). The inoculated plants produced typical symptoms of rice blast disease on the leaves of IRRI-6. The fungus produced typical mycelial growth on PDA medium when re-isolated from small pieces of inoculated leaves of IRRI-6 variety.

After 15 days of inoculation, 80% disease incidence with 25.8% average disease severity was recorded in *M. oryzae* inoculated plants, whereas, disease was not developed in un-inoculated plants. Inoculated plants showed 78% colonization by *M. oryzae* as compared to un-inoculated plants (Fig. 4).

Fig. 1. Disease symptoms, (a) whitish or grayish specks along the leaf margins, (b) elongated and diamond shape spots and (c) large lesion on the leaf. (d) large elongated lesions on stem and (e) brown diamond shaped spots on rice seeds.
Fig. 2. Fungi isolated from leaves of different rice varieties

Fig. 3. Fungi isolated from seeds of different rice varieties.
Discussion

In the present studies, total seven fungi viz., *Magnaporthe oryzae*, *Curvularia lunata*, *Helminthosporium oryzae*, *Fusarium moniliforme*, *Alternaria alternata*, *Nigrospora oryzae* and
Aspergillus niger were isolated from the leaves and seeds of five rice varieties namely, IRRI-6, IRRI-8, DR-82, KS-282 and Shua. N. oryzae was isolated only from IRRI-8 variety. M. oryzae was the most predominant fungus that was isolated from leaves and seeds of all rice varieties. The infection of M. oryzae varied with the rice varieties IRRI-6 appeared to be the highly susceptible variety followed by KS-282 to M. oryzae, whereas minimum infection was observed on Shua. All other fungi such as, C. lunata, H. oryzae, F. moniliforme, A. alternata, N. oryzae and A. niger were isolated in comparatively with low frequencies. Our studies showed strong association of M. oryzae with affected plant parts of all rice varieties. The results are in accordance to those reported by other workers such as Naeem et al., 19 also recorded Pyricularia oryzae, Alternaria padwickii, Curvularia sp., Fusarium moniliforme and Bipolaris oryzae from seeds, shoot and root of different rice varieties. Similarly, Khan et al., 14 isolated F. semitectum, F. moniliforme, F. oxysporum, A. alternata, A. padwickii, C. oryzae, C. lunata, Drechslera oryzae and P. oryzae from rice seeds. Similar findings have been mentioned by Bhutta and Hussain 4, Whaid et al., 29, Misra and Dharam 18 and Mew and Gonzales 17.

Inoculated healthy rice plants of IRRI-6 variety by M. oryzae produced typical symptoms of the disease. The leaves of plants inoculated with M. oryzae showed whitish to grayish specks initially, which enlarged and became spindle shaped necrotic spots with brown to reddish brown margins within 15 days of inoculation. M. oryzae appeared as the virulent and aggressive pathogen on the IRRI-6 variety, the disease appeared on 80 % of the inoculated plants after two weeks inoculation with 25.8 % average disease severity. It clearly indicated that blast disease caused by M. oryzae is one of the destructive diseases of rice and can cause severe damage and yield reduction under favourable environment conditions on susceptible variety. In the present studies the infection of M. oryzae also caused 25.5% and 55.4% reduction in plant height and plant weight, in inoculated plants as compared to un-inoculated plants. There are several reports described that M. oryzae can infect rice plant at any growth stage and caused considerable losses (Mew and Gonzales 17; Webster and Gunnell 31; Sirithunya et al., 27 and Phinyarat et al., 20). Similarly, Anthony 2 reported that 10-30 % of world rice crop was disturbed by blast disease caused by M. oryzae. In Nepal, rice blast caused by the same fungus is considered as the most serious disease of rice and caused 50% reduction in the yield on susceptible varieties. Similarly, Singh 25 reported 60-80 % yield losses from India on widely grown rice variety due to M. oryzae infection.

References


