Study on Bakanae disease of rice and evaluation of cultivars in Gilan and Zanjan provinces, Iran.

Hossein Saremi¹ and Farhang Farrokhi²

1- Department of plant pathology, Faculty of Agriculture, Zanjan University, Zanjan, Iran. Phone: +98-241-Saremih@mail.znu.ac.ir; Email: Saremih@mail.znu.ac.ir
2- Zanjan technology Incubator (ZTI), Zanjan, Iran. Phone: +98-241-5152676 Email: Farrokhi@mail.znu.ac.ir

Abstract
Crown and root rot of rice is one of the important fungal diseases of rice in Gilan and Zanjan provinces, Iran. Plants and soil around the roots of infected rice plants were collected and used to identify the causal agent. Root and crown parts were surfaced sterilized with sodium hypochlorite and then cultured on PDA, PPA and CLA media. Soil samples prepared in water agar were used to isolate the pathogen. The causal agent was identified Fusarium moniliforme as a soil borne fungus. Colonies were initially white but turned violet to grey late. Microconidia were arranged in chain and macroconidia were cylindrical and long with 3-5 septa. The disease was severe in Zanjan province particularly along Ghezel Ozan river where the infection ranged from 70-80%. Root and crown rot was more prevalent in areas where Champa and Gerdeh were being cultivated continuously. Persistent cultivation of rice and seed sowing method intensified disease development and caused significant economic losses. Binam cultivar was the best resistant to the disease in studied areas.

Keywords: Crown, Fusarium moniliforme, Resistance, Rice, Root, Rot

Introduction
Rice (Oryza sativa L.) is the most important agricultural crop of north in Iran. Root rot and crown rot is a significant disease of rice and caused most yield losses in various parts of the country. Generally the increased incidence and economic importance of the disease has been linked to environmental conditions such as humidity and use of susceptible varieties. The disease acquires economic proportions in the northwest of Iran, especially in Gilan and Zanjan provinces. It is also a serious problem in other countries including Japan, Taiwan and Thailand (Nelson et al. 1981). Bakanae disease of rice, also called foot rot in India, occurs widely in Asia and sporadically in other areas of rice production. The most common symptom of disease in some countries may be the elongation of the plant stems. However, the disease may be called “Bakanae” which is a Japanese word meaning bad seedlings (Sun and Snyder, 1981). In addition the disease has been reported from the rice tracts of South Asia and European countries (Saremi, 2000). It has been reported that several soil- borne fungi are involved in causing crown rot of rice in Iran. For example, Khosravei (1999) isolated different species of seed borne fungi especially Fusarium species from the affected rice fields in the Mazandran province. Crown and root rot of rice usually causes yield losses under wet conditions in spring when infection of the crown or stem tissue occurs near the soil surface. The present study has been carried out to assess the occurrence of root and crown rot disease of rice by F. moniliforme and the contribution of factors on the increase of its incidence in northwest Iran.

Materials and methods
Sample collection
The study covered rice fields in the Gilan and provinces from 1999 to 2004. All these areas were visited and plants with symptoms growth reduction, crown necrosis, white heads and
stem elongation were collected and transferred to the laboratory of plant pathology in Zanjan University. Different samples infected with crown rot and root rot were collected from each province each year to isolate and identify the causal agents. Various fractions of the samples i.e. roots, crowns, and soils around the roots were cultured in different media.

**Incubation**

All cultures were incubated in a room lighted with near-ultraviolet wave lengths (black light tube, Philips TL 36 w/80 RS F40 BLB) and fluctuating temperatures regime, 25°C during day and 20°C in night under 12 h photoperiod. Sporulation and pigmentation of *Fusarium* species are favored by this situation (Burgess et al., 1994). Soil dilution technique was also used to isolate inoculums from soil in the root zone suspect to be infected with crown rot disease.

**Media**

Collected samples were cultured in PDA (Potato, Dextrose, and Agar) as common medium, PPA (Peptone, PCNB, and Agar) as selective medium and CLA (Carnation, Leaf, and Agar) as natural medium after surface sterilization with sodium hypochlorite. Cultures were kept in light room with fluctuating temperatures (25 °C during day and 20 °C at night) and UV lights (12 h light and 12 h dark) to allow the colonies of the pathogen to grow (Burgess et al. 1994). The soil dilution method (Saremi 1998) was used to isolate pathogen inoculum from the soil around the roots of the wheat plant suspected to infected crown rot disease. Pure culture was obtained from each isolate using the single spore culture method and all isolates were identified (Burgess et al. 1994; Saremi 1998).

**Soil dilution plate technique**

The technique involved the uniform dispersion of 1 ml of soil suspension of infected field across a selective medium such as PPA. Actually, one gram of infected soil was added to the water agar to produce soil suspension. Propagules in the soil sample suspension germinated within 2-3 days on PPA and produced small colonies by 5-7 days. The suspension was uniformly dispersed over the medium by carefully pipetting 1 ml of soil suspension onto the medium on one edge oft the PPA. The plate was then held with a slight slope away from the suspension and gently shaken at right angles to the slope. The suspension slowly spread across the plate with a uniform wetting front.

**Pathogenicity test**

Pathogenicity test was occurred in naturally infected field in Zanjan province. The population density of causal agent was high, with 1575 colony forming propagules unite in one gram soil (CFU g –1 in 0 to 10 cm depth) in some studied areas. By the way, ten varieties of rice including Binam, Kadous, Shafagh, Sahel, Fajr, Khazar, Neda, Nemat, Gerdeh and Champa were cultured in naturally infected soil. Rice cultivars were also cultured in nearly non infected field to obtain the effect of reduction in population density of the fungus to yield loss. The population density of *F. moniliforme* in this field was low with 145 CFU g –1 which nearly healthy crop can be grown. The rate of infection and yield loss production of both fields were generally compared to determine the relatively resistant or sensitivity of varieties in studied area.

**Results**

**Symptom of disease**

Generally various fungal species produced different symptoms on the infected rice plants. However, the main symptoms of common root rot and crown rot in the areas studied were yellowing, stem elongation, pink coloration around the crown, and white head caused by *F. moniliforme* (Fig.1a). The diseased plants were mostly stunted and the symptoms were most
striking near or below the surface. They include brown spots, blotches and rotting on the crown and roots. Some of the infected seeds had not growth and showed rot before emergence in different locations (Fig 1b). This sort of disease was mostly severed in areas that seeds were cultured instead of using seedlings such as growers in Zanjan province. Other soil-borne fungi such as *Rhizoctonia solani* were also found in rare situations. The fungus leads to formation of white heads with little or no grain. The disease appears when the fungus is able to build up sufficient inoculum in the soil over two or more years on susceptible varieties.

**Morphology of causal agent**

During the 4-years study in different rice fields various fungal isolates were identified. All the soil-borne fungi that cause common root rot and crown rot were mostly isolated from the studied fields. The incidence of crown rot was more in the relatively humid locations in Zanjan and Gilan provinces. These two humid provinces especially Gilan are close to the Caspian Sea and receive heavy rainfall in the crop season (April and May). The main pathogen was *F. moniliforme* that formed floccose mycelium and became grayish violet or grayish magenta with age (Fig 2a). Microconidia were formed abundantly in chains from monophialide on branched conidiophores or from monophialide formed directly on the hyphae (Fig. 2b).

**Nearly resistance varieties**

Crown rot disease on rice resulted in poor seed filling, leading to significant yield losses. Plants of infected fields were compared with noninfected plants. Investigation showed that there were differences in the extent of yield loss in different areas (Fig 3). Inoculum density of *Fusarium moniliforme* could affect the incidence of disease. High population density of the pathogen has been associated severity of crown rot and yield production of rice in studied area. “Binam” had the most yield production and less infected disease and “Champa” showed low yield production in the field (Fig 3). However, the result showed there was an obvious difference between the infection rate and yield loss of varieties in studied area. Binam was the main resistant race in the field, however Kadous and Shafagh also showed better resistance than Nemat, Neda and Khazar races. Other varieties such as Fajr, Sahel and Shafagh were relatively resistant to the disease, any how they showed reduced crop production.

**Rate of yield losses**

Root rot and crown rot disease caused white heads and resulted in poor seed filling, leading to significant yield losses. Plants of infected fields were compared with noninfected plants in each rice cultivar. Investigation showed that there were differences in the extent of yield loss in different cultivars. Binam had lower infection (%7) but Champa (local name) showed the highest (%38) infection in natural infected soil (Tab. 1).

**Discussion**

Commonly, the incidence of crown rot and root rot of rice were significantly affected by environmental factors, mainly rainfall and susceptible varieties. The effect of climate, especially rainfall and temperature, on the abundance of *Fusarium* species has been reported by various investigators (Sangalang et al. 1995; Burgess et al. 1996; Marasas et al., 1988). The incidence of *Fusarium moniliforme* in humid areas supported the contention that *F. compactum* was isolated only from warmer sites and *F. sambucium* was recovered only from temperate to cold areas (Saremi et al. 1999).
It is important to develop resistant cultivars through breeding effort by applying recurrent selection. It was obvious that using susceptible cultivar such as “Gerdeh” and “Champa” (local name) resulted in high epidemic crown rot disease in Zanjan province.

Some physiological and morphological characters in rice may also associate with disease resistance. Study also showed that some varieties with partial resistance had less reduction in yield due to light infection. For example, Binam, Kadous, Shafagh and Sahel varieties were more resistant than other varieties such as Namat, Neda and Khazar. Namely, more resistant ones should be cultivated over large areas to increase high production.

There was an investigation by Padasht et al. (1996) to find suitable fungicide for controlling crown rot disease on rice in north Iran. Of course chemical control may have some negative effect on other soil microorganisms or produce resistance of the fungal pathogen. However, it has been reported that Initial greenhouse studies showed the treatment of infested seed with the fungicide Maxim resulted in a significant reduction of diseased plants in some places (Nyvall, 1999). Further study, especially field tests, are needed to determine the effectiveness of fungicide seed treatments under field conditions, possible phytotoxicity resulting in inhibition of root elongation, and potential impact on stand establishment.

Since the disease was described as widespread, occurring in various counties and also spreading to worldwide locations. Scientists took a deeper look into the life cycle of fungus caused crown rot disease on rice in different countries to find the best way to control the pathogen.

However the incidence of crown rot disease on grains caused by *Fusarium* species was mostly correlated with stubble management as the fungus survives in the infected residues (Wearing and Burgess 1977). Burgess et al. (1993) determined the consistency of the effects of stubble management on crown rot disease over longer periods of continuous crop cultivation and on the increase of disease incidence. The seedlings of rice may become infected from the pathogen *Fusarium moniliforme* in soil or residue but all available information indicates the disease is primarily seed born. The pathogen is abundant in residue of harvested plants, providing the main source for infestation of seed. Studies have shown that the pathogen is primarily a surface contaminant of seed.

It was observed that in the Zanjan province the development of crown rot disease was more in poor soils than in soils rich in organic matter content. It has been generally reported that the soil-borne pathogens are less than fungal saprophytes in the soil with high organic matter content (Van Bruggen, 1995). However, crown rot was more on deep heavy clay soils with continuous rice cultivation.

Since crown rot and root rot of rice have become major diseases in the areas of continuous rice cultivation in Iran, particularly in the northern rice belt, it is important to control them through management practices and use of tolerant cultivars. Crop rotation, stubble burnt in autumn and careful selection of nearly resistant varieties can be helpful to minimize the incidence of disease. We have to stop the using continuously one variety such as “Gerdeh” or “Champa” which leads to significant increase in the incidence of crown rot caused by *F. moniliforme* in Zanjan province.

**Acknowledgments**
The study was supported by the Iranian Center of National Projects under the project No 21205 "Crown rot and root rot diseases of rice and its ecological control in northwest of Iran" which gratefully acknowledged.

References:
4- Khosravei, V. 1999 Investigation on main seed borne fungal disease of rice in north Iran, Ms thesis, Agricultural College Tehran University, 102 pp.
Tables:

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Yield, g/1 m²</th>
<th>Yield loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy crop</td>
<td>Diseased crop</td>
</tr>
<tr>
<td>Binam</td>
<td>341</td>
<td>317</td>
</tr>
<tr>
<td>Kadous</td>
<td>285</td>
<td>253</td>
</tr>
<tr>
<td>Gerdeh</td>
<td>356</td>
<td>245</td>
</tr>
<tr>
<td>Champa</td>
<td>345</td>
<td>214</td>
</tr>
</tbody>
</table>

**Table 1**: Yield losses in four cultivars of rice crop in Zanjan province, Iran caused by *F. moniliforme* in natural infected soil.

**Figure Legends**

**Figure 1**: Elongation and white head (a) and seed rot of rice (b) caused by *Fusarium moniliforme* (b) in Zanjan province.

**Figure 2**: Colony morphology on PDA medium (a) and long chain of Microconidia (b) of *Fusarium moniliforme* the casual agent of rice rot.
Figure 3: Cultivation of different rice varieties in infected soil (a) and white head disease due to infection by *F. moniliforme*.