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Binam a Rice Cultivar, Resistant for Root Rot Disease on Rice Caused by *Fusarium moniliforme* in Northwest, Iran

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Abstract: Rice is a major crop in different parts of Northwest Iran especially in Zanjan province. Survey in cultivated rice in fields showed the plants were severely subjected to root rot and white head disease which caused yield losses in rice growing regions. A natural project was prepared from 2004 to 2006 to get general information on the causal agent of disease and its control management. Infected plants were collected from different studied areas and transferred to laboratory. Crown and plant roots were cultured in PDA as common media and PPA as selective media for *Fusarium* species after surface sterilization. Plates were incubated in standard culture room then isolated fungi were identified. Different *Fusarium* species were isolated , however the main pathogen isolated from plant samples and soil around the roots was *Fusarium moniliforme* Sheldon. The disease caused up to 75% yield losses in some fields in studied areas. The disease was more prevalent in areas where susceptible varieties were being cultivated continuously. Study showed the Binam cultivar was the main resistant to the disease and had the most yield production in the field.

Key words: Binam, resistance, rice, root rot, *Fusarium moniliforme*

INTRODUCTION

Generally, rice (*Oryza sativa* L.) comprises a main imperative agricultural crop in North Iran (Saremi, 2005). Improving its production is one of the main goals for reaching more food security and alleviating recurrent food shortages in different parts of the continents (Allarangaye *et al*., 2007). Root rot and crown rot is a significant disease on rice and caused most yield losses in various part of the rice grown areas. The prevalence and economic importance of disease has been linked to culturing susceptible varieties and environmental conditions particularly moisture. The disease acquires economic proportions in the northwest of Iran, especially in Gilan and Zanjan provinces (Khosravei, 1999; Saremi, 2000). It is also a serious problem in other countries including Japan, Taiwan and Thailand (Webster and Gunnell, 1992; Kini *et al*., 2002; Sharifi-Tehrani *et al*., 2004; Saremi, 2005).

The most common symptoms 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 (Groth *et al*., 2004). Bakanae disease of rice, also called foot rot in India, occurs widely in Asia and sporadically in other areas of rice production. In addition the disease has been reported from the rice tracts of south Asia, European countries and America (Desjardins *et al*., 2000; Saremi, 2000). The classic and most conspicuous symptom of the disease is the hypertrophy effect or abnormal elongation of plant. However, these symptoms can even be observed from a distance. The affected plants may be several inches taller than normal plants, thin, yellowish green and may produce adventitious roots at the lower nodes of the culms. Diseased plants bear few tillers and leaves dry up quickly. The affected tillers usually die before reaching maturity; when infected plants survive, they bear empty panicles (Webster and Gunnel, 1992; Heong *et al*., 2005). The disease is consistent problem on much of the April planted rice and on certain minimum tillage fields. While reducing stand considerably, rice yields may be unaffected by seedling diseases if the stand is uniform, because modern rice varieties have the ability to tiller to fill available space and compensate for early stand loss.

Symptoms of bakanae become visible about a month after planting. Infected seedlings appear to be taller, more slender and slightly chlorotic when compared to healthy seedlings (Fig. 1a). The rapid elongation of infected plants is caused by the pathogen's production of the plant hormone, gibberellin. Plants with bakanae are often visible arching above healthy rice plants, infected plants senesce early and eventually die before reaching maturity. If they

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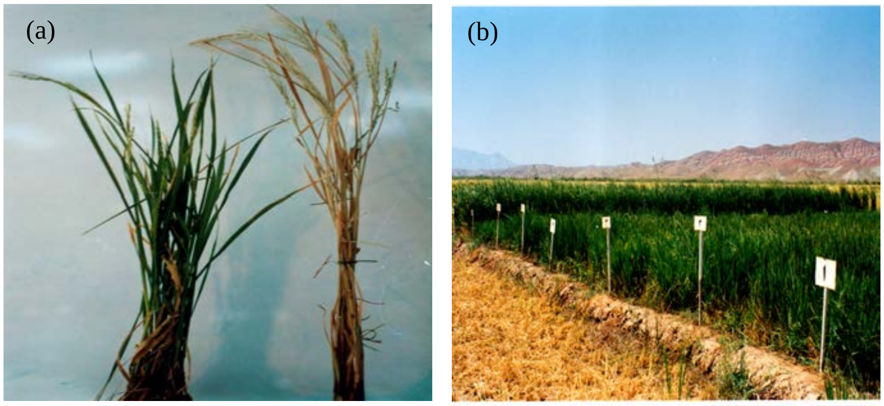

Fig. 1: Stem elongation of rice caused by *Fusarium moniliforme* (a) and culturing ten rice cultivars on natural infected field in Zanjan province (b)

do survive to heading, they produce mostly empty panicles. Generally, Bakanae is one of the oldest known diseases of rice in Asia and it produced by gibberellic acid in some parts of world (Kim, 1981; Groth *et al*., 2004). The disease normally cause yield reduction and economical damage, so researchers try to find different way to manage it even resistant cultivar (Zhang *et al*., 2005).

As diseased plants senesce and die, mycelium of the fungus may emerge from the nodes and may be visible above the water level. After the water is drained, the fungus sporulates profusely on the stems of diseased plants (Saremi, 2005). The sporulation appears as a cottony mass and contaminates healthy seed during harvest. The bakanae pathogen can over winter as spores on the coat of infested seeds. It can also over winter in the soil and plant residue. However, infested seed is the most important source of inoculums.

Generally, the main agricultural crops, including wheat, maize and rice, are common in northwest Iran. Previous studies showed they were mostly infected with various *Fusarium* species (Saremi, 2000). By the way, a wide range of toxic compounds such as mycotoxins produced by *Fusarium* species. Fore example, moniliformin production in food and feed products has been reported from *Fusarium moniliforme*, *F. nygamai*, *F. oxysporum*, *F. avenacelum*, *F. acuminalum* and *F. equiseti* world wide (Sagaram and Shim, 2007; Rheeder *et al*., 2002; Nelson *et al*., 1993). We have isolated *F. moniliforme* from rice plants and animal feeds in the Zanjan Province many times. It has been reported that moniliformin may cause myocardial necrosis in animals which is similar to a serious heart disease observed in some regions of China (Jestoi, 2008; Flaherty and Woloshuk, 2004). However, the profile of secondary metabolites produced by most *Fusarium* species is still unclear (Maragos *et al*., 2008).

Since root rot and white head or empty panicles of rice have become major diseases in the areas of continuous rice cultivation in Iran, particularly in northwest country, it is important to control the disease by means of tolerant cultivars. Normally, careful selection of nearly resistant varieties can be helpful to reduce the incidence of disease. The present study had been carried out to assess the causal agent of root rot and wilting disease on rice and become aware of resistance variety to this main disease in Northwest Iran.

MATERIALS AND METHODS

Sample collection: Rice grown fields in the Gilan and Zanjan provinces were visited and infected plants were collected from 2004 to 2006 years. Infected plants showing white heads, stem elongation and root rot were selected and transferred to the laboratory of plant pathology in Zanjan University. Generally, samples were put in paper packet to reduce humidity for decreasing bacterial grown, then samples were dried and prepared for culturing in media. Various parts of samples including roots, stems and heads were cultured in different media for growing colonies of the pathogen and estimation fungal pathogens.

Media: Normally, samples were cultured in Potato Dextrose Agar (PDA) as common medium and peptone, PCNB, agar (PPA) as selective medium after surface sterilization with sodium hypochlorite. The cultures were incubated in a room lighted with standard conditions with fluctuating temperature regime. Daily temperature was between 25 and 20°C and in night under 12 h photoperiod and intermittent black UV light tube. The soil dilution method (Saremi, 2005) also was used to isolate pathogen inoculums from the soil around the roots of the wheat plant suspected to be infected with 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).

Incubation: Fungal 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. Generally, sporulation and pigmentation of *Fuasrium* species are privileged by this situation (Burgess *et al*., 1994). The method of soil dilution was used to isolate inoculums from soil in the root region, suspected to be infected with root rot disease.

Soil dilution plate technique: This technique concerned the uniform dispersion of 1 mL of soil suspension of infected field across a selective medium such as PPA. In fact, 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 1 week. Commonly, the suspension was uniformly dispersed over the medium by carefully pipetting 1 mL of soil suspension onto the medium on one edge of 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 (Saremi, 2005).

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 (cfu g^{-1}) in one gram soil of the natural infected field in some studied areas. By the way, ten rice varieties including Binam, Kadous, Shafagh, Sahel, Fajr, Khazar, Neda, Nemat, Gerdeh and Champa were cultured in the naturally infected soil in Zanjan province (Fig. 1b). Rice cultivars were also cultured in nearly non infected field to obtain the effect of 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 the fields.

RESULTS

Symptom of disease: The main symptoms of the diseased plants caused by *Fusarium moniliforme* were white head; stem elongation, coloration around the crown and root rot in the fields (Fig. 2a). 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 roots. Some of the infected seeds had not growth and showed decay before emergence in some locations (Fig. 2b). This sort of disease was mostly severed in areas that seeds were cultured as alternative culturing seedlings in Zanjan province. The fungus guided to formation of white heads with little or no grain. The disease appeared when the fungus was able to build up sufficient inoculums in the soil over two or more years on susceptible varieties.

Morphology of causal agent: Through the three years study in different rice fields various fungal isolates were identified. Other fungi that cause common root rot and white head on rice were mostly isolated from the studied fields. The frequency of the disease was more in the relatively rainfall locations in Zanjan province. How ever,

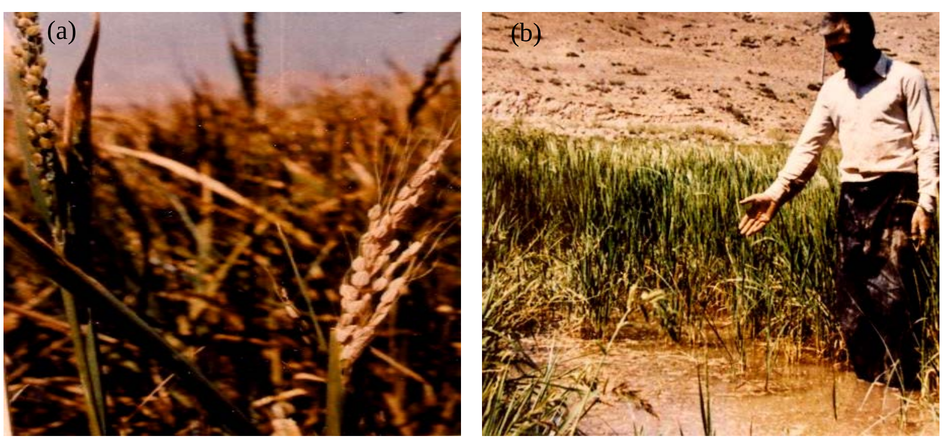

Fig. 2: White head (a) and seed rot of rice due to infection by *Fusarium moniliforme* in Zanjan province (b)

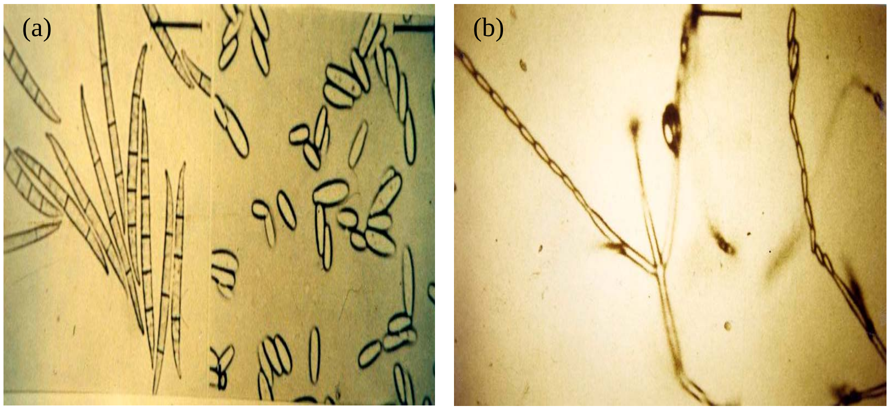

Fig. 3: Macroconidia and Microconidia of *Fusarium moniliforme* (a) and long chain of microconidia (b) isolated from infected rice

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

	Yield (kg/10 m^2)		
Cultivars	Healthy crop	Diseased crop	Yield loss (%)
Binam	3.41	3.17	7
Kadous	2.85	2.53	11
Gerdeh	3.56	2.45	31
Champa	3.45	2.14	38

the main pathogen was *F. moniliforme* which had microconidia abundantly in chain and nearly long macroconidia with 4 to 6 cells (Fig. 3a, b).

Nearly resistance varieties: Generally the root rot disease on rice produced poor seed filling, leading to significant yield losses. Plant yield productions from infected field were compared with yield in no infected plants. Investigation showed that there were apparent differences with the extent of yield loss in different locations (Table 1). Inoculums 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.

Consequently, Binam cultivar had the most yield production and less infected disease, while other cultivars especially Champa showed low yield production in the field. The result showed there was an obvious difference between the infection rate and yield loss of varieties in studied area. The Binam showed the main resistant to the disease than other cultivars. However, Kadous and Shafagh cultivars also showed better resistance than Nemat, Neda and Khazar. Other varieties such as Fajr, Sahel and Shafagh were relatively resistant to the disease any how they showed reduced yield production.

Rate of yield losses: Yield production of rice plants cultured in infected fields (each 10 m^2) were compared with yield of no infected plant cultivars. Production of one plate field namely Yield 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 (Table 1). Actually, traditional cultivars were sensitive to the disease and had low economical yield production.

DISCUSSION

The incidence of root rot disease on rice was significantly affected by environmental factors and susceptible varieties. Generally, effect of climate on the abundance of *Fusarium* species has been reported by various investigators (Häberle *et al*., 2007; Desjardins *et al*., 2000; Saremi *et al*., 1999; Burgess *et al*., 1993). 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; Sangalang *et al*., 1995). However, *F. moniliforme* was occurred in northwest Iran and caused main disease on rice. Because the disease is an economically importance in the country we it is necessary to find the best way for its control. The seedlings of rice may become infected by *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

The mono-cultivation of susceptible rice cultivar in studied area made soils highly suppressive to the root rot disease. Actually population density of *Fusarium moniiforme* was low in non cultivated soils or in non cultivated crop soils. Normally, *Fusarium* population occurs in low density in non cultivated soils or in field where only resistant plants had been grown (Naeimi *et al*., 2003). On the other hand study showed the population density *Fusarium* species was high in the field cropped to one plant for the previous 15 years. Population can be reduced in rotation but repeated rice cultivation increased the pathogen population and yield losses. The study showed the annual yield losses of rice were 40 to 70% in Zanjan province at 2004 and 2006 years.

Generally, 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 introduce the suitable way to control the fungal pathogen (Robertson-Hoyt *et al*., 2000; Fiume and Fiume, 2003; Sha *et al*., 2007; Häberle *et al*., 2007). 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 traditional cultivars resulted in high epidemic crown rot disease in Zanjan province. Some physiological and morphological characters in rice may also associate with disease resistance (Iqbal *et al*., 2005; Kristi *et al*., 2006). Study also showed that some varieties with partial resistance had loss reduction in yield due to light infection. For example, Binam, Kadous, Shafagh and Sahel varieties were more resistant than other varieties such as Nemat, Neda and Khazar. Namely, further resistant cultivars should be cultivated over large rice grown areas to increase high yield production.

There was an investigation by researchers to find suitable fungicide for controlling crown rot disease on rice in North Iran and other countries (Ogawa, 1988; Padasht *et al*., 1996). 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 resulted in a significant reduction of diseased plants in some places (Nyvall, 1999). Further studies, especially field tests, are needed to determine the effectiveness of fungicide seed treatments under field conditions. By the way, the chemical treatment has negative side on environment and economical problems for growers.

The effect of antagonist on bakanae disease could be noticed since it has been reported that plants from treated rice seeds with *Pseudomonas solanacearum* were healthier and greener than those from non treated seeds (Rosales *et al*., 1986). This could be due to suppression of initial inoculums in the seeds by seed soaking in bacterial suspension, since *Fusarium moniliforme* is seed-borne. This could also be due to detoxification of fusaric acid, a phytotoxin produced by *F. moniliforme* as shown by researchers (Toyoda *et al*., 1988; Sharifi-Tehrani *et al*., 2004; Kamilova *et al*., 2006).

Actually, mycotoxin production by the *Fusarium* species is also one major factor that has stimulated international research on this fungal genus. Commonly, a wide range of toxic compounds is produced by *Fusarium* species but the profile of secondary metabolites produced by most *Fusarium* species is still unclear. The process of biosynthesis of these compounds or the extent of contamination of the food chain is still not fully understood. However, *F. moniliforme* may produces a group of mycotoxins especially fumonisins on infected rice plants. This mycotoxin cause considerable health and economic concerns for humans and animals worldwide which must be notified (Desjardins *et al*., 2000; Brown *et al*., 2006; Sagaram and Shim, 2007).

Actually, discovery of a growing number of naturally occurring *Fusarium* mycotoxins with serious threat to human and animal heath caused worldwide interest in toxicogenic *Fusarium* species. Developments in mycotoxicology have also led to the discovery of secondary metabolites in *Fusarium* species (Summerell *et al*., 2001; Teichert, 2006). These findings are based on weight loss and death of cattle and sheep in some parts of Iran. The cattle and sheep were occasionally fed with cereals contaminated with Fusarium species, particularly in the Zanjan Province (Saremi and Okhovvat, 2006).

In view of the fact that root rot and white head of rice have become major diseases in the rice grown areas in Iran, particularly in Zanjan province, it is essential to manage it by using tolerant cultivars. Off course, collection the nearly resistant variety can be helpful to minimize the incidence of disease. On the other hand, using continuously just one conventional variety should be prevented, since it leads to significant increase of the disease. We tried to select a rice resistant cultivar to a soil bore pathogen *F. moniliforme* which cause root rot and wilting on rice plants in northwest the country. Study showed, Binam, was a resistant cultivar to the root rot disease on rice and produce good quality yield in the field. Result of this project direct rice growers to make use of the nearly resistant rice cultivar for managing the incidence of root rot disease on rice and have high production.

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