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RESEARCH ARTICLE

PATHOGENICITY OF *FUSARIUM ANTHOPHILUM* AND *FUSARIUM FUJIKUROI* ASSOCIATED WITH BAKANAE DISEASE OF RICE: A LANDMARK STUDY UNDERTAKEN IN SOUTH AFRICA

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ABSTRACT

This article has been extracted from the PhD thesis of the principal author, who submitted the study to the University of South Africa in the year 2013. Pathogenicity of three representative isolates of each *Fusarium anthophilum* (MRC 5519, MRC 5520 and MRC 5806) and *Fusarium fujikuroi* (MRC 5807, MRC 5808 and MRC 5809) obtained from naturally infected rice plants in the fields with bakanae symptoms were tested. All isolates of both *F. anthophilum* and *F. fujikuroi* were able to cause bakanae symptoms on seedlings of four different rice cultivars and lines. This is the first report of the occurrence of bakanae disease of rice in South Africa and both *F. anthophilum* and *F. fujikuroi* are new records as pathogens of rice in South Africa. *Fusarium anthophilum* has not been reported before from any country in the world as a pathogen of rice (*Oryza sativa*) and as a causative fungus of bakanae disease.

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INTRODUCTION

South Africa is not a classical rice growing country and rice was introduced as a crop by Taiwanese experts who were brought into the apartheid homelands around 1976, and subsequently these homelands were incorporated into democratic South Africa, post 1994, at the advent of South African democracy and some of them like the North West, Northern Cape, Free State and Mpumalanga Provinces are rice growing areas which made up some of the newly formed Provinces of the Republic. After the departure of the Taiwanese rice experts the rice industry virtually collapsed and recently there has been a move to revive rice growing on a commercial basis in order to look at the possibilities of exports and to alleviate poverty and hunger in a country that has extremes of unemployment, inequality and poverty. South Africa has immense potential to grow rice and therefore rice research assumes huge significance within the country.

In the first 4 – 5 years of rice growing in the North Western areas of South Africa, no fungal diseases were reported. Due to on – going cultivation of rice without crop rotation, disease started to develop and farmers complained about disease problems with rice. There was an outbreak of bakanae disease in the fields of North West Province and Kuruman area of the Northern Cape Province during the 1988 – 1989 crop seasons. The most evident symptom of the disease is yellowing and abnormal elongation of infected rice seedlings due to gibberellic acid production by bakanae causal agent and this led to the Japanese name bakanae, meaning 'foolish seedlings' (Ou, 1985; Amatulli *et al.*, 2010). The diseased rice plants developed adventitious roots from the lower nodes. Bakanae caused by *F. fujikuroi* (teleomorph: *Gibberella fujikuroi*) is well known as important disease of rice, especially at the seedling stage. It has been reported from most rice producing countries such as Japan (Ito and Kimura, 1931), India (Thomas, 1931), Philippines (Reyes, 1939), Thailand (Kanjansoon, 1965), Bangladesh (Mia and Zaman, 1973), Spain (Marin – Sanchez and Jimenez – Diaz, 1982), Turkey

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(Copcu and Karaca, 1983), China (Yang *et al.*, 2003), Italy (Amatulli *et al.*, 2010) and Pakistan (Butt *et al.*, 2011; Ghazanfar *et al.*, 2013). It was reported by Ito and Kimura (1931) that the disease caused a 20% percent yield loss in Hokkaido. Yield losses were reported as high as 40–50 percent in Kinki – Chugoku Region of Japan (Anonymous, 1975). Pavgi and Singh (1974) reported losses of up to 15 percent due to bakanae disease in the eastern districts of the State of Uttar Pradesh in India. Grain yield losses of up to 40 percent due to bakanae disease have been reported by others (Maragos *et al.*, 1997; Desjardins *et al.*, 2000). In various rice growing countries, losses due to bakanae disease could be higher than 70 percent (Iqbal *et al.*, 2011).

Desjardins *et al.* (2000) reported that bakanae disease of rice can be caused by one or two *Fusarium* species. *Fusarium fujikuroi*, *F. proliferatum* and *F. verticilloides* (*F. moniliforme*) have been reported to cause bakanae disease of rice (Wuff *et al.* 2010; Heng *et al.*, 2011). Bakanae disease caused by *F. moniliforme* (*F. verticilloides*) has become a major limiting factor in rice production throughout the world (Ghazanfar *et al.*, 2013). *Fusarium anthophilum* (MRC 5519, MRC 5520, and MRC 5806) and *F. fujikuroi* (MRC 5807, MRC 5808 and MRC 5809) have been isolated from naturally infected rice plants showing bakanae symptoms in the fields of the North West and Northern Cape Provinces of South Africa. There could be multiple causes of the bakanae disease since different species of *Fusarium* are known to cause the disease.

Objectives of the study

The objectives of this research study were to:

- Establish the pathogenic nature of isolates of *F. anthophilum* (MRC 5519, MRC 5520 and MRC 5806).
- Establish the pathogenic nature of isolates *F. fujikuroi* (MRC 5807, MRC 5808, and MRC 5809) in four different rice cultivars and lines.

MATERIALS AND METHODS

Collection of rice seeds and inoculation procedures

Rice seeds of Hsinchu 56, Chainan 8, Lemont and IRRI 52287 – 15 were used and collected from the provincial Department of Agriculture and Rural Development, North West Province and from the Grain Crops Institute of the Agricultural Research Council of South Africa, situated in Potchefstroom in the North West Province of South Africa. Inoculum for the pathogenicity trial was obtained by washing conidia from 10 days old cultures on PDA plates of the test isolates of *F. anthophilum* (MRC 5519, MRC 5520 and MRC 5806) and *F. fujikuroi* (MRC 5807), MRC 5808, and MRC 5809), the resultant conidial suspensions were concentrated by centrifugation at 4000 rpm for 20 minutes and re-suspending the pellets in sterilized water so that each suspension contained 125×10^3 spores/ml (Ahmed *et al.*, 1986). Pathogenicity of isolates was tested on seedlings of the cultivars / lines Hsinchu 56, Chainan 8, Lemont and IRRI 52287 – 15. Rice seeds were pre-soaked in cold water for four hours and soaked again in hot water at 54 degrees C for 15 minutes to eliminate external micro-

organisms. The hot water treated seeds were then dried at room temperature for future use.

The hot water treated seeds were soaked for 48 hours. Total 40 sprouted seeds of each cultivar / line were inoculated with each test isolate of *F. anthophilum* (MRC 5519, MRC 5520 and MRC 5806) and *F. fujikuroi* (MRC 5807, MRC 5808 and MRC 5809) by immersing them in 1ml inoculum for 5 minutes in a test tube. Ten inoculated seeds were individually planted in a plastic pot of 12.5 cm diameter containing sterilized sand. For each isolate, 16 potted seedlings were grown for treatment and 16 potted seedlings were similarly grown but not inoculated and used as control. The seedlings were grown in a growth chamber with 14 hours per day photo period of 15 000 lux fluorescent light. Temperature and relative humidity ranged from 29 degrees to 25 degrees C and 70 to 90 percent respectively in the growth chamber (Marin – Sanchez and Jimenez – Diaz, 1982). A half strength Hoagland nutrients solution (Dhingra and Sinclair, 1995) was used to provide nutrients for growth of rice seedlings. The pots were arranged in a randomized block design with four replications (Mead and Curnow, 1987; Box *et al.*, 2005). Final counts of diseased and healthy seedlings were made when they were 28 days old.

Statistical Analysis

The pathogenicity tests of three isolates of each of *F. anthophilum* (MRC 5519, MRC 5520, and MRC 5806) and *F. fujikuroi* (MRC 5807, MRC 5808 and MRC 5809) on seedlings of four rice cultivars / lines Hsinchu 56, Chainan 8, Lemont and IRRI 52287 – 15 were measured and statistically analyzed by using the Least Significant Difference (LSD) as an Analysis of Variance (ANOVA), as well as cluster analyses. The LSD was calculated at $p > 0.05$. Data analysis was performed using STATISTICA software (Statsoft Inc.; version 17.0). The raw sets were expressed as a percentage of diseased plants. This percentage was used for the rest of the analyses. For each isolate, the final disease index was calculated as the mean of the four pots in each of the four repetitions. Cluster analysis was performed to assign treatment combinations similar to each other into groups.

RESULTS

In pathogenicity tests, of all representative isolates of both *F. anthophilum* (MRC 5519, MRC 5520 and MRC 5806) and *F. fujikuroi* (MRC 5807, MRC 5808 and MRC 5809) were able to cause bakanae symptoms on seedlings of four different rice cultivars and lines. The diseased seedlings showed abnormal growth of stems and development of adventitious roots from lower nodes. The diseased rice plants showed dark brown to black roots. Typical bakanae symptoms appeared, 14 days after seeds sowing only in seedlings of four test rice cultivars inoculated with all the isolates of both *F. anthophilum* and *F. fujikuroi*. The bakanae symptoms were not seen on any of the control rice plants. All the pathogenic isolates of *F. anthophilum* and *F. fujikuroi* were re-isolated from diseased seedlings showing bakanae symptoms. All the four test rice cultivars were susceptible to the isolates of *F. anthophilum* and *F. fujikuroi*. The disease incidence caused by isolates of *F. anthophilum* (MRC 5519, MRC 5520 and MRC

5806) varied from 70 to 95 percent and the disease incidence caused by *F. fujikuroi* (MRC 5807, MRC 5808 and MRC 5809) varied from 78 to 100 percent on a 0 – 100 disease index scale. The isolates of *F. anthophilum* such as MRC 5519 caused disease incidence from 80 to 83 percent; MRC 5520 caused disease incidence from 70 to 85 percent and isolate MRC 5806 caused disease incidence from 80 to 95 percent on a 0 – 100 disease index scale. The isolates *F. fujikuroi* such as MRC 5807 caused disease incidence from 98 to 100 percent, MRC 5808 caused disease incidence 78 to 93 percent and MRC 5809 caused disease incidence from 85 to 88 percent on a 0 – 100 disease index scale. In this study, all isolates of *F. anthophilum* (MRC 5519, MRC 5520 and MRC 5806) and *F. fujikuroi* (MRC 5807, MRC 5808, and MRC 5809) were found as causal pathogen of bakanae disease of rice. None of the test cultivars / lines was found resistant to the disease caused by *F. anthophilum* and *F. fujikuroi*. The cluster analyses were performed to assign treatment combinations similar to each other into groups and analyses clearly showed treatment combinations similar to each other into few groups.

DISCUSSION

Fusarium anthophilum and *F. fujikuroi* were isolated from diseased rice plants showing bakanae symptoms in the fields of North West and Northern Cape Provinces of South Africa. All the isolates of *F. anthophilum* and *F. fujikuroi* caused bakanae disease in four different rice cultivars and lines in artificial inoculation of pathogenicity tests. None of the test rice cultivars was found resistant to the disease caused by isolates of *F. anthophilum* and *F. fujikuroi*. The isolates of *F. fujikuroi* were ranked based on their pathogenicity and rated between 78 to 100 percent on the 0 – 100 disease index scale. The isolates of *F. anthophilum* were ranked on the basis of their pathogenicity and rated between 70 to 95 percent on the same scale. Cluster analyses on different rice cultivars and lines clearly showed the treatment similar to each other into groups. These groupings were the indication of intrinsic genetic differences in rice cultivars and lines. The environment had little influence on the variables, because the experiments were conducted under a controlled environment.

The bakanae disease showed complex of disease symptoms including root and crown rot, abnormal elongation of stems, wilting, stunting and the formation of adventitious roots at nodes on the lower portions of stems (Yamanaka and Honkura, 1978; Sun and Snyder, 1981; Ou, 1985; Webster and Gunnell, 1982). The bakanae disease was discovered 100 years ago in Japan but, it seems the species causing diseases are known but not exhaustive as yet (Amatulli *et al.*, 2010).

The causal organism of bakanae disease was described in Japan as *Gibberella fujikuroi* (Sawada) (Ito and Kimura, 1931). The anamorph was considered to be *Fusarium moniliforme* (*F. verticillioides*) (Saccardo) Nirenberg by Booth (1971). But Nirenberg (1971) differentiated it as a separate species, *Fusarium fujikuroi*. Nelson *et al.* (1983) did not accept *F. fujikuroi* as a separate species but included it in *F. moniliforme* as the “short chained” type of *F. moniliforme*. Marasas *et al.* (1986) reported the presence of polyphialides in *Fusarium* isolates from rice with bakanae disease (“bakanae strains”) and

excluded them from *F. moniliforme*. These authors concluded, however, that the use of the name *F. fujikuroi* sensu Nirenberg (Nirenberg, 1976) for these cultures would create the problem of separating this species from *F. proliferatum* (Matsushima) Nirenberg only on the basis of the host plant (= rice). On the basis of studies on the ultrastructure of collarette formation. Tiedt and Jooste (1988) concluded that *F. fujikuroi* could be clearly differentiated from moniliforme, but not from *F. proliferatum*. Prof WFO Marasas who confirmed the identity of two *Fusarium spp*; expressed concern that these three isolates identified as *F. fujikuroi* could not be differentiated morphologically from *proliferatum*. Thus these three isolates could either be called “*F. fujikuroi* (because they were isolated from rice with bakanae disease). Or short – chained strains of *F. proliferatum*. In this study and according to current understanding of the taxonomy of this fungus, two *Fusarium* species *F. anthophilum* and *F. fujikuroi* caused bakanae disease in South Africa. Desjardins *et al.* (1997) reported the isolation of some strains of *F. fujikuroi*, *F. proliferatum* and *F. verticillioides* from bakanae infected rice seedlings from various geographic areas. Bakanae disease of rice is caused by one or more *Fusarium* species (Desjardins *et al.* (2000).

The results of this study on the pathogenicity of *F. anthophilum* and *F. fujikuroi* isolated from rice plants showing bakanae symptoms in the fields of North West and the Northern Cape Province of South Africa corroborate the conclusion of Desjardins *et al.* (2000). *Fusarium fujikuroi*, *F. proliferatum* and *F. verticillioides* have been reported to cause bakanae disease of rice (Wuff *et al.* 2010; and Heng *et al.* 2011). Amatulli *et al.* (2010) reported the isolation of few *Fusarium* species such as *F. fujikuroi*, *F. proliferatum*, *F. verticillioides*, and *F. equiseti* from bakanae diseased rice plants and seeds in Italy. In pathogenicity tests only *F. fujikuroi* isolates caused bakanae disease. Carter *et al.* (2008) reported *Fusarium fujikuroi* as the causal pathogen of bakanae disease of rice (*Oryza sativa*) water grass (*Echinochloa oryzoides*) O. Bolos Masclans) and barnyard grass (*Echinochloa crus – gali* L. Beauv) in California in the USA. Zainudin *et al.* (2008) reported the isolation of *F. fujikuroi*, *F. proliferatum*, and *F. verticillioides*, *F. sacchari* (E.J. Butler and Hafiz Kahn) *W. Gams* and *F. subglutinans* (Wollenw and Reinking) P. E. Nelson, Toussoun and Marasas from bakanae diseased rice plants in Malaysia and Indonesia. However, in pathogenicity tests, only the isolates of *F. fujikuroi* caused bakanae disease.

Conclusion

This is the first report of the occurrence of bakanae disease of rice in South Africa. Both *F. anthophilum* and *F. fujikuroi* are new records as pathogens of rice in South Africa, although Gorter (1977) reported “*Fusarium moniliforme*” / *Gibberella fujikuroi*” to be associated with foot rot of rice in South Africa. Marasas *et al.* (1987) did not report *F. anthophilum* in South Africa but later the fungus was isolated from oats in South Africa (Thiel *et al.*, 1991). *Fusarium anthophilum* has been isolated from Copra in Trinidad (Gordon, 1956). *Fusarium anthophilum* (as moniliforme var. *anthophilum*) has been reported to produce substances with gibberellin – like properties (Gordon, 1960; Marasas *et al.* 1984). *Fusarium*

anthophilum has been reported to reduce the height of wheat seedlings (Mantecon et al., 1984) in Argentina. It is concluded in terms of this study that *F. anthophilum* has not been reported before in any country in the world as pathogen of rice (*Oryza sativa*) or as causative fungus of bakanae disease. This is a landmark study conducted in South Africa which is an emerging rice growing nation and therefore the research conducted in respect of *Fusarium anthophilum* and *Fusarium fujikuroi* associated with bakanae disease of rice makes a significant contribution to rice cultivation and research in South Africa, in fact its contribution to the world cannot be underestimated.

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