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ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN: 0976-3376

Asian Journal of Science and Technology
Vol. 6, Issue 06, pp. 1523-1532, June, 2015

RESEARCH ARTICLE

THE MYCOFLORA ASSOCIATED WITH DISEASED PLANTS AND SEEDS OF ORYZA SATIVA (RICE): EXEMPLIFYING THE IMPORTANCE OF EFFECTIVE DISEASE CONTROL MANAGEMENT

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ARTICLE INFO

Article History:
Received 25th March, 2015
Received in revised form
30th April, 2015
Accepted 17th May, 2015
Published online 29th June, 2015

Key words:
Management,
Mycoflora,
Diseased Plants,
Rice,
Seeds,
Pathogenic,
Toxigenic,
Fungi,
Quarantine.

ABSTRACT

Various Mycoflora (fungi) were isolated from diseased rice plants and rice seeds within some rice growing regions of South Africa. The isolates of various fungi were initially identified on the basis of their morphological characteristics and the identification of the representative isolates of Fusarium spp. were confirmed based on the DNA sequence of the translation elongation factor 1 –a (TEF – 1-a) gene. A total of six species of Fusarium were identified namely, F. anthophilum, F. chlamydosporum, F. compactum, F. equiseti, F. fujikuroi and F. semitectum. This is the first report regarding the Fusarium species from rice in South Africa. Fusarium anthophilum has not been found associated with bakanae disease of rice in South Africa. Fusarium anthophilum has not been found to be associated with bakanae disease of rice from any country of the world before. Fungi other than Fusarium spp. were also isolated and identified only on the basis of their morphological characteristics. A total of eight other species of fungi were identified namely, Alternaria alternate, Alternata longipes, Cochliobolus miyabeanus, Nigrospora sphaerica, Phoma eupyrena, Phoma jolyana, Phoma sorghina and Pithomyces sp. It is the first report regarding A. alternate, A. longipes, N. sphaerica, P. eupyrena, P. jolyana and Pithomyces sp. from rice in South Africa. This paper therefore looks at salient issues concerning rice, in order to contribute to the science of rice cultivation. The paper underscores the importance to identify pathogenic and toxigenic fungi correctly for effective disease control management, quarantine purposes and as a basis for making decisions to protect agricultural crops as well as other natural resources according to Rossman and Palm – Hernandez (2008) and Heng et al (2011). South Africa is made up of Nine Provinces and this research straddled four of the provinces. It is in these four provinces that the bulk of South Africa's small production of rice is grown. Experts from Taiwan during the days of the homeland Bantustans (1976) were brought into these homelands particularly Bophuthatswana which included parts of today's Northern Cape Province, the North West Province and, parts of today's Free State Province to grow rice and vegetables and, to explore fish farming opportunities. The departure of the Taiwanese experts before the collapse of the homeland system precipitated the virtual collapse of the rice growing opportunities. The Mpumalanga province was not part of this arrangement but, it overlapped into at least two of today's provinces. Given the shortage of food worldwide and extreme levels of poverty in South Africa, it might be a feasible idea for the South African government to intensify the possibilities of growing rice in some provinces that have past experience in this regard. The importance of research in rice can therefore, not be under – estimated in the agricultural dynamic of South Africa and must be further explored.

INTRODUCTION

Rice belongs to the family of Gramineae and the genus Oryza sativa is native to tropical and subtropical Southern Asia, while the African rice, Oryza glaberrima is native to West Africa Habib *et al.,* 2012). Improved varieties of rice (Oryza sativa) were introduced in the regions of the North West Province, Free State Province, Northern Cape Province, Mpumalanga Province and Kwa – Zulu Natal Province of South Africa by Taiwanese experts in the 1980's. Yields of up to 8 – 12 tonnes / ha paddy rice were obtained from some areas (Billette, 1986; Dreyer, 2004).

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Bakanae disease symptoms were observed in the rice fields in the North West Province and the Kuruman area of the Northern Cape Province during the 1988 – 1989 crop seasons. The disease symptoms observed were yellow and abnormal elongation of infected rice seedlings due to gibberellic acid production by the bakanae causal agent (Copco and Karaca, 1983; Ou, 1985; Amatulli *et al.,* 2010; Iqbal *et al.,* 2011). In various rice growing countries, losses by disease could be 70 percent (Ito and Kimura, 1931; Ou, 1985; Iqbal *et al.,* 2011). The disease causes both quantitative and qualitative losses with severe losses under field conditions and the disease is able to attack the rice plant from pre – emergence to flowering (Ou, 1985; Iqbal *et al.,* 2011).

The bakanae disease has become a major limiting factor in rice production throughout the world (Ghazanfar *et al.,* 2013). It is therefore vitally important to identify pathogenic and toxigenic fungi correctly for purposes of effective disease control management, for quarantine purposes and as a basis of making decisions to protect agricultural crops as well as other natural resources (Palm – Hernandez, 2008; Heng *et al.,* 2011).

MATERIALS AND METHODS

Field Surveys

Disease surveys were conducted to determine the fungal species associated with various plant parts of diseased rice plants in the field. The surveys were repeated at three growth stages of rice plants, namely as seedlings – tillering, elongation – booting and ripening in the same fields. Disease infected rice plant samples were collected during three growing seasons (1988 / 1989, 1989 / 1990, 1990 / 1991). Samples were collected from rice fields at six rice projects situated in the regions of the North West Province, Free State Province and Northern Cape Province of the Republic of South Africa. During each survey forty rice fields were sampled in each growing season. Five sample areas were randomly selected in each field by throwing a wire counting square I meter in diameter. Samples were collected from the fields and isolation made within the shortest possible time from each symptom type recorded.

Objectives of the Investigation

The objective of this investigation was to isolate and identify various pathogenic and toxigenic fungi associated with different plant parts of diseased rice plants in the fields of the North West Province, Free State Province and Northern Cape Province during the crop seasons outlined above and, from rice seeds from the Mpumalanga Province during the crop seasons of 1995 / 1996 with the support of the established protocols by the Department of Agriculture in the region. The researcher did not intend to isolate all possible micro – organisms that can occur on rice such as the well-known toxin producing Aspergillums and Penicillium species. Since many of these fungi are saprophytic; it can be expected that vast numbers of the species would be found. These organisms are not known to be pathogens of rice.

ISOLATION

Isolation of fungal species from diseased rice plants

Isolations of fungal species were done from diseased plant parts by direct plating of plant tissues. The affected tissues namely roots, stems and leaves were washed under running tap water and the surface was sterilized with a 1 percent sodium hypochlorite solution (NaOCl) for one minute and rinsed twice in sterilized water. Small pieces of affected tissues were placed in a 9 cm petri dish containing potato dextrose agar (PDA) and incubated at 25 plus / minus 1 degree C in darkness for up to 10 days or until sufficient growth or spores enabled isolation. Single spore pure cultures were obtained and stored on PDA slants for identification (Martin – Sanchez and Jimenez Diaz, 1982; Copcu and Karaca, 1983; Nelson *et al.,* 1983; Leslie and Summerel, 2006).

Isolation of fungal species from rice seeds

Seed samples of six lowland rice cultivars / lines (TK5, TK6, TK7, TK9, and TC10, and USA 201) were obtained from a rice project at Bushbuckridge in Mpumalanga Province to determine fungi associated with rice seeds in the areas. The seeds were the harvest of 1995 / 1996 crop season. The researcher was unable to visit the rice project to collect disease infected rice plants during the growing seasons. Therefore, rice samples were obtained to determine the fungi associated with rice seeds in the areas. A total of 400 seeds were selected at random and used for each seed sample. Fungi associated with rice seeds were isolated using two methods namely, the blotter method and potato dextrose agar (PDA) method (ISTA, 1985; Mew and Misra, 1984). The blotter method involved placing 20 non – sterilized seeds on three layers of Whatman's 9 cm blotter (Whatman No. 1) in a 9 cm petri dish moistened with sterile water. The plateswere incubated at 25 plus / minus 1 degree C for 8 days in an alternating cycle of 12 hours light and 12 hours darkness (ISTA, 1985; Mew and Misra, 1984). The PDA method involved plating of 20 surfaced sterilized seeds from each sampled cultivar / line on to petri dishes and subsequent incubation at 25 plus / minus 1 degree C for 8 days in an alternation of cycles of 12 hours light and 12 hours of darkness. Single spore cultures were obtained and stored on PDA slants for later identification.

Identification of fungal isolates based on morphological characteristics

Fusarium strains isolated from diseased rice plants and rice seeds were initially identified on the basis of the morphological species concepts of Gerlach and Nirenberg (1982) and Nelson et al. (1983). Morphological species concepts are based on similarity of observable morphological characters, for examp0le, size of spore and shape of spore (Summerell *et al.,* 2003). Cultural characters of fungal species were assessed morphol9ogically through examination using a stereomicroscope and a compound microscope with reference to Booth (1971, 1977), Gerlach and Nirenberg (1982) and Nelson et al. (1983). The identification of Fusarium spp; based on morphological features, were further correlated with the descriptions of Leslie and Summerell (2006). Cultural characters of fungi other than Fusarium were assessed morphologically by examination using a stereomicroscope and a compound microscope with reference to Ellis (1971), Carmichael et al. (1980), Sutton (1980), Ellis and Ellis (1985) and Barnett and Hunter (1998) for morphological identification.

Identification of Fusarium isolates on the basis of molecular characters

DNA Extraction and Amplification

Isolates were grown on PDA at 25 degrees C for 7 days. Deoxyribonucleic acid (DNA) was isolated using the DNA easy plant mini extraction kit (Qiagen, Valencia, CA) by following the manufacturers protocol after mycelium placed in Eppendorf tubes and ground with ca. 10ug sterile, chemically treated sand. The extracted DNA was used as a template in the polymerase chain reactions (PCR).

The part of the TEF gene was amplified using the primer set EF1 '(5' – CGAATCTTTGAACGCACATTG – 3)' EF2 (5' – CCGTGTTTCAAGACGGG – 3') (O'Donnell *et al.,* 1998). The PCR reaction consisted of 1 x Dream Taq reaction buffer with MgCl2, dNTPs (250uM each), primers (O.2uM each), and template DNA 9 (25ng) and Dream Taq polymerase (O.5U). The PCR reaction conditions, for the TEG gene region was amplified by initial denaturation at 94 degrees C for 2 minutes. This was followed by 30 cycles of denaturation at 94 degrees C for 1 minute, annealing at 52 degrees C for 1minute, and then elongation at 72 degrees C for 1 minute and elongation step at 72 degrees C for 5 minutes. The resulting PCR amplicons were purified using a QIAquick PCR purification kit (QIAGEN, Hilden, Germany).

DNA Sequencing and Sequence Comparisons

DNA sequences were determined from PCR amplicons using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit AmpliTaq DNA Polymerase (Applied Bio - systems, Warrington, UK) using the primers EF1 and EF2. Sequences generated in this study were deposited in GenBank. The partial sequence data for translation elongation factor 1 –a gene was compared against both the NCBI (GeneBank) database and the Fusarium Database (Geiser *et al.,* 2004). Reference sequences were selected on the basis of the BLAST (Basic Local Alignment Search Tool) results and previously published phylogenetic relationships within the Fusarium incarnatum – equiseti species complex (FIESC). DNA sequences were aligned using a multiple sequence alignment programme, MAFFT (Katoh *et al.,* 2002). MAFFT is a novel method for rapid multiple sequence alignment based on Fast Fourier Transform (FFT). Gaps were treated as missing data in the subsequent analysis. Phylogenetic analysis was based on parsimony using PAUP 4.O (Phylogenetic Analysis Using Parsimony and Other Methods Version 4; Swofford, 2002). Heuristic searches were done with random addition of sequences (100 replicates), tree bisection – reconnection (TBR) branch swapping, and MULPARS effective and MaxTrees set to auto increase. Phylogenetic signal in the data sets (g1) was assessed by evaluating tree length distributions over 100 randomly generated trees (Hillis and Huelsenbeck, 1992(. The consistency (C1) and retention (R1) indices were determined for the TEF data set. Phylogenetic trees were rooted with Fusarium oxysporum Schlechtend. Emend. Snyder and Hansen as monophyletic sister out group to rest of the taxa. Bootstrap analyses were performed to determine branching point confidence intervals (replicates) for the most parsimonious trees generated for the TEF data set.

RESULTS

Field surveys

Bakanae disease

Diseased rice plants showed symptoms of abnormal elongation of stems and were several centimeters taller than healthy plants in the fields. The diseased plants were thin, pale and yellowish green. The diseased rice plants developed adventitious roots from the lower nodes. The crown tissues were so rotten that the root system could be separated easily from the culms. The diseased rice plants died at the booting stage, showing dark brown to black roots and erect panicles with no kernels. These symptoms were similar to bakanae disease of rice. Bakanae disease symptoms were found in the rice fields at Manyeding near Kuruman in the Northern Cape Province, Bodibe near Itsoseng, Dinokona near Zeerust, Moiletsoane near Odi and Taung in the North West Province

Sheath rot

Diseased rice plants showed sheath rot symptoms in the rice fields. The symptoms were usually found at late booting stage, the upper part of the leaf sheaths were found affected. Early symptoms were oblong to irregular brown to black spots. The centre of the spot became greyish white with brown margins. Sheath not lesions enlarged and affected the entire leaf sheath.Brown to black discolouration was seen occasionally on the rice culms. In case of severe infection, panicles were found partially exerted. Partially emerged panicles produced poorly filled grains. Sheath rot symptoms were found in the rice fields at Manyeding near Kuruman in the Northern Cape Province, Bodibe near Itsoseng, Dinokana near Zeerust, and Moiletsoane near Odi in the North West Province and Woodbridge near ThabaNchu in the Free State Province.

Brown spot

Brown spot symptoms were found in both surfaces of rice plants. Symptoms of brown spots were also found on the leaf sheaths and stems. The spots were oval in shape and relatively uniform and fairly evenly distributed over the leaf surface. The spots were found as brown with grey or whitish centers when fully developed. The center of the lesion became straw coloured but the margin remained dark brown. Brown spot symptoms were found on leaves of both young and adult plants. The brown spot disease symptoms were found in the rice fields of at Dinokana near Zeerust in the North West Province and Manyeding near Kuruman in the Northern Cape Province.

Minute leaf spot

Rice plants showed characteristic symptoms of numerous minute dark brown or black lesions on the leaves. Rice plants also showed necrotic symptoms on the leaves. Minute leaf spot symptoms were found in the rice fields at Manyeding near Kuruman in the Northern Cape Province, Dinokana near Zeerust and Bodibe near Itsoseng in the North West Province.

Isolation and Identification of Different Species of Fungi

The different species of fungi were isolated and identified from diseased rice plants in the fields and rice seeds in South Africa. Representative cultures of the various species were deposited in the culture collection of PROMEC, Medical Research Council (MRC) at Tygerberg, in the Western Cape Province of South Africa and, the duplicate cultures were later deposited in the National Collection of Fungi, Culture Collection of the Plant Protection Research Institute (PPRI), Agricultural Research Council of South Africa, for long time storage and for future use.

Isolation and Identification of Fusarium Isolates on Morphological Characteristics

A total of 6 morphologically known Fusarium species such as F. anthophilum, F. chlamydosporum, F. compactum, F. equiseti, F. fujikuroi and F. semitectum were isolated from diseased rice plants and rice seeds.

- **Fusarium anthophilum:** (MRC 5519, MRC 5520 and MRC 5806) was isolated from diseased rice plants with bakanae symptoms from the rice fields in the North West and Northern Cape Provinces. Diseased rice plants showed abnormal elongation of stems and the plants were thin, pale and yellowish green. The diseased rice plants developed adventitious roots from the lower nodes. The crown tissues were found seriously rotten. The diseased rice plants died at the booting stage and showed dark brown to black roots and erect panicles with no kernels. This Fusarium species produced dense aerial mycelium with abundant microconidia of oval globose and pear shaped on potato dextrose agar medium. Microconidia were borne on polyphialides. Chlamydospores were absent.
- **Fusarium chlamydosporum:** (MRC 7368) was isolated from a seed sample from the rice cultivar TK9 from the Lowveld area of Mpumalanga Province. The fungus produced characterized abundantaerial mycelium from white to pink to brown in colour. The fungus produced spindle shaped microconidia, which were formed on polyphialides.
- **Fusarium compactum:** (MRC 7369, MRC 7370) was isolated from a seed sample from the rice cultivar TC 10 from the Lowveld area of the Mpumalanga province. Fusarium compactum produced mycelium with white to greyish rose in colour on potato dextrose agar medium. The fungus produced thick walled, shorter, fatter and compact microconidia on potato dextrose agar medium and produced abundant Chlamydospores. The fungus did not produce microconidia.
- **Fusarium equiseti:** (MRC 5817, MRC 5818 and MRC 5819) was isolated from diseased rice plants with symptoms of sheath rot from rice fields in the North West, Free State and Northern Cape Provinces. The diseased rice plants showed sheath rot symptoms at booting stage. The upper most part of leaf sheaths were found affected. The early symptoms were oblong to irregular brown to black spots. The center of the spot became greyish white with brown margins. Sheath rot lesions enlarged and affected the entire leaf sheath. The rice culms showed brown to black discolouration. The panicles of the diseased rice plants were found partially exerted and partially emerged panicles produced poorly filled grains. The fungus Fusarium equiseti produced abundant aerial mycelium of white to brown in colour on PDA. Chlamydospores were abundant. Macroconidia were variable in size and shape and strongly curved or bent with elongated apical cells.
- **Fusarium fujikuroi:** (MRC 5807, MRC 5808 and MRC 5809) was isolated from the diseased rice plants with bakanae symptoms from the rice fields in the North West and Northern Cape Provinces. Diseased rice plants showed symptoms of abnormal elongation of stems and the plants were found thin, pale and yellowish green. The diseased rice plants developed adventitious roots from the lower ends. The crown tissues were found seriously rotten. The diseased rice plants died at the booting stage and showed dark brown to black roots and erect panicles with no kernels. Fusarium fujikuroi was characterized by dense white mycelium with abundant ovoid to clavate shaped microconidia in chains and in false heads on monophialides and polyphialides on potato dextrose agar medium. The fungus did not produce Chlamydospores.
- **Fusarium semitectum:** (MRC 7363, MRC 7364, MRC 7365, MRC 7366, and MRC 7367) was isolated from seed samples from the rice cultivars TK5, TK7 and TK9 from the Lowveld area of Mpumalanga Province. Fusarium semitectum produced abundant uniform mycelium that varied from white to pink in colour on PDA medium. The microconidia were not produced. Macroconidia were produced in sporodochia and were strongly curved with an elongated apical cell.

Identification of Fusarium Isolates Based on Molecular Characteristics

- DNA extraction and amplification Aplicons of the TEF gene region were 640 bp in size.
- DNA Sequencing and Sequence Comparisons

The partial sequence data for the translation elongation factor 1 – a (TEF) was compared against the NCBI (Gene /Bank) database, the Fusarium database (Geiser *et al.,* 2004) and Fusarium MLST data (O'Donnell *et al.,* 2009) to confirm the identity of isolates of Fusarium species isolated from diseased rice plants and rice seeds. All the strains of Fusarium species isolated from diseased rice plants and rice seeds were confirmed as Fusarium spp. by comparing three Fusarium identification databases. The results of these independent analyses were summarized.

Parsimony analysis of the TEG gene region was done to determine the phylogenetic placement of the Fusarium equiseti rice isolates. The TEF data set by inserting gaps resulted in a total of 587 characters used in the comparisons of the different species. All the parsimony uninformative and constant characters were excluded, resulting in 185 parsimony informative characters. Heuristic searches on the data set generated one hundred most parsimonious trees. In the TEF data set, the rice isolates of F, equiseti grouped together within the Fusarium incarnatum – equiseti species complex (FIESC). The South African isolates from rice were clustered together in a single clade with the F. equiseti and F. incarnatum isolates forming two separate sub – clades. The South African isolates clustered with none of the 28 phylogenetic lineages in the FIESC (O'Donnell *et al.,* 2009). The South African F. equiseti rice isolates proved to present a new phylogenetically distinct species in this complex.

Isolation and Identification of other Fungi

Fungi other than Fusarium species were isolated from diseased rice plants and rice seeds and identified only on the basis of their morphological characters. The species identified were, Alternaria alternata, Alternaria longipes, Cochliobolus miyabeanus, Nigrospora sphaerica, Phoma eupyrena, Phoma sorghina and a species Pithomyces. These are discussed hereunder:

- **Alternaria alternate:** was isolated from diseased rice leaves showing characteristic symptoms of numerous minute dark brown or black lesions and it was isolated from rice projects of the North West and Northern Cape Provinces. The fungus was also isolated from a seed sample from the rice cultivar TK5 of the Lowveld area of Mpumalanga Province. The fungus produced mycelium that was usually black or grey in colour. The conidiophores were borne singly or in small groups, simple or branched and could be straight or flexuous. Conidia were formed in long, branched chains and obclavate, obpyriform, oboid or ellipsoidal in shape with short conical or cylindrical beak.
- **Alternaria longipes:** was isolated from a seed sample from the rice cultivar TK5 of the Lowveld area of the Mpumalanga Province. The fungus produced amphigenous type of mycelium. Condiophores were borne singly or in groups, erect or ascending simple or loosely branched. Conidia were solitary and were in chains obclavate, rostrate, pale to mid pale brown.
- **Cochliobolus miyabeanus:** was isolated from diseased rice plants showing brown spot symptoms. Dark brown spots were found on both leaf surfaces. The brown spots were also found on leaf sheath and stems. It was isolated from the rice projects of North West and Northern Cape Provinces. The fungus was also isolated from the rice seed samples from the rice cultivar TK7 and USA 201 of the Lowveld area of Mpumalanga Province. The conidiophores of the fungus were solitary or in small groups, which might be straight, flexuous or geniculate and were pale to mid brown or olivaceous brown. Conidia were curved, navicular, fusiform or obclavate and cylindrical in shape.
- **Nigrospora sphaerica:** was isolated from a seed sample of the rice cultivar TK6 of the Lowveld area of Mpumalanga Province. The fungus produced shining white mycelium colonies. Hyphae were creeping, short and vaguely branched and produced brown to black conidia. Conidia were borne on aspices of small branches, perfectly globose in shape.
- **Phoma eupyrena:** was isolated from seed samples from the rice cultivar USA 201 of the Lowveld area of Mpumalanga Province. The fungus produced aerial mycelium that is dark brown to olivaceous green to brown grey in colour. The fungus produced Chlamydospores with pale brown colour. Conidia were found cylindrical or ellipsoid, straight or slightly curved in shape, biguttulate and aseptate.
- **Phoma jolyana:** was isolated from seed samples of the rice cultivars TK5, TK7, Tk9 and TC10 of the Lowveld area of Mpumalanga Province. The fungus produced abundant fruit bodies on hyphae (pycnidia). Conidia were ellipsoid or slightly irregular in shape and biguttulate. The Chlamydospores were terminal and lateral.
- **Phoma sorghina:** was isolated from diseased rice plants with sheath rot symptoms. Short linear and brownish lesions were found on the leaves and leaf sheaths of diseased rice plants. The upper most part of leaf sheaths were found affected. Sheath rot lesion enlarged and affect6ed the entire leaf sheath. It was isolated from rice projects of the North West and Northern Cape Provinces. The fungus was also isolatedfrom seed samples of rice cultivars TK5, TK7. Tk9 and TC10 of the Lowveld area of Mpumalanga Province. The fungus produced fluffy to dense aerial mycelium which was grey – green to olivaceous or darker, but with a characteristic white to salmon pink tinges. Conidia were found ellipsoid in shape and eguttulate. Chlamydospores were single celled.
- **Pithomyces sp:** was isolated from seed samples of the rice cultivar TK7 of the Lowveld area of Mpumalanga Province. The fungus produced black mycelium colonies. The fungus produced conidia with mid to dark brown, echinulate or verruculose. Mostly with 3 transverse and 1 or 2 longitudinal septa, each with a protruding fractured denticle at the base.

DISCUSSION

In this study, different species of fungi such as Fusarium and other fungi were isolated from diseased rice plants showing various disease symptoms in the rice fields of North West, Northern Cape, and Free State Provinces and isolated from rice seed samples from the Mpumalanga Province of South Africa. It is therefore necessary to outline by verification in the literature to support the discussion and conclusions of the findings in respect of the different Fusarium species and other Fungi. A brief description and discussion is undertaken hereunder to support the research undertaken in this study as follows:

Fusarium Species

Six species of Fusarium were isolated from diseased rice plants and rice seeds and identified (Species described and mentioned earlier) on the basis of morphological species concepts of Gerlach and Nirenberg (1982) and Nelson et al. (1983). Morphological identification of Fusarium species provides a great deal of information but the system may not suffice for a complete identification (Summerell et al. 1983). Morphological identification of representative strains of Fusarium species isolated from diseased rice plants and rice seeds in this study, were therefore, confirmed with DNA sequences of the translation elongation factor 1 – a (TEF) was compared against the NCBI database (GenBank), the Fusarium database (Geiser et al. 2004) and Fusarium MLST database (O'Donnell et al. 2009). The identification of strains of Fusarium species based on Fusarium ID and MLST database largely correlated with morphological identification, whereas Gene/ Bank database did not provide significant insight to the identification of the strains but at least confirmed all the strains as Fusarium spp. in the F. incarnatum – equiseti species complex and Gibberella fujikuroi species complex. The identification of all strains of Fusarium species isolated and identified morphologically from diseased rice plants and rice seeds were confirmed as Fusarium species by comparing three Fusarium identification databases. In parsimony analysis of the TEF gene, the isolates of F. equiseti from rice grouped together within the Fusarium incarnatum – equiseti species complex (FIESC). The South African Isolates of F. equiseti from rice were clustered together in a single clade with the F. equiseti and F. incarnatum isolates forming two separate sub – clades. The South African rice isolates of F. equiseti clustered with none of the 28 phylogenetic lineages in the FIESC (O'Donnell *et al.,* 2009). The South African F. equiseti rice isolates proved to present a new morphological distinct species in this complex.

Fusarium anthophilum and F. fujikuroi

Fusarium anthophilum and F. fujikuroi were isolated from the diseased rice plants showing bakanae symptoms in the rice seedbeds and in the main rice fields of North West and Northern Cape Provinces. Fusarium fujikuroi is a well-known fungus isolated from rice plants in association with bakanae disease of rice (Kanjanasoon, 1965; Ou, 1985; Desjardins *et al.,* 2000; Carter *et al.,* 2008; Zainudin *et al.,* 2008; Amatuli et al' 2010; Wuff et al. 2010; Heng et al. 2011). This is the first report of bakanae disease of rice in South Africa and both F. anthophilum and F. fujikuroi have been isolated for the first time from rice plants in association with bakanae disease of rice in South Africa. However, Gorter (1977) reported "F. moniliforme /G. fujikuroi" to be associated with foot rot of rice in South Africa, Fusarium anthophilum has been isolated from cultivated and wild rice in association with headblight of rice in Minnesota, USA (Nyvall *et al.,* 1999). It must be stated with confidence that F. anthophilum has not been reported before from any country in the world, in association with bakanae disease of rice. The association of two Fusarium spp. Such as F. anthophilum and F. fujikuroi with bakanae disease of rice in South Africa will complicate the control measures of the disease. Moreover both F. anthophilum and F. fujikuroi produce multiple mycotoxins (Desjardins, 2006) and would increase the risks of contamination of rice grains with mycotoxins in the fields and in the stores of South Africa.

Fusarium chlamydosporum and F. compactum

In this study, Fusarium chlamydosporum and F. compactum have been isolated from a rice seed sample from the rice cultivar TK9 and Tk10 respectively from the Lowveld area in the Mpumalanga Province of South Africa. Fusarium chlamydosporum has also been isolated from rice seeds in India (Nath *et al.,* 1970), in Nepal (Desjardins, et al. 2000) and in Argentina (Broggi and Molto, 2001). Previously, Marasas et al. 1987) confirmed the isolation of F. chlamydosporum from various crop plants such as maize, sorghum and pasture plants in South Africa. Because of its association with major food grains including rice and because of its ability to produce toxins, Desjardins (2006) suggested that risk assessment for F. chlamydosporum will be necessary. This is the first report of the isolation of F. chlamydosporum from rice in South Africa. Fusarium compactum has also been isolated from rice in Nigeria (Somorin Bankole, 2010), from maize in South Africa (Marasas and Van Rensburg, 1986; Desjardins, 2006. The fungus has been isolated from wheat in South Africa (Van Wyk *et al.,* 1987) and from wheat kernels in the Slovak Republic (Rochacik and Hudec, 2005). The fungus has been reported to produce trichothecenes and fumonisins (Cole et al. 1988; Desjardins, 2006; Lezar and Barros, 2010). Therefore, there are risks of contamination of rice grains with mycotoxins produced by the fungus in the stores of South Africa. This is the first report of isolation of F. compactum from rice in South Africa.

Fusarium equiseti and F. semitectum

In this study, F. equiseti has been isolated from diseased rice plants showing sheath tot symptoms in the fields of warm climates in the North West and Northern Cape Provinces and in the fields of the Free State Province, where the climate is relatively cool.

Fusarium equiseti has been isolated from the diseased rice plants with sheath rot symptoms in India (Kang and Rattan, 1983). Fusarium equiseti has been isolated from adult rice plants showing a discolouration in the vascular tissues of the culm (Martin – Sanchez and Jimenez – diaz, 1982). The fungus has been reported as a seed borne pathogen of rice in India (Singh and Khare, 1983) and in Nigeria (Reckhause and Adamon, 1986). The fungus has been reportedto produce trichothecenes (T@), zearalenone and moniliformin (Hussein, 1991). There are risks of contami9nation of rice grains in the stores of South Africa. This is the first report of isolation of F. equiseti from rice in South Africa. Fusarium semitectum has been isolated from rice seeds in India (Nath *et al.,* 1970; Singh and Khare, 1983; Saini, 1985), from rice seeds in Malawi (Siddiqi, 1980), from rice seeds in Thailand (1994) and from rice seeds in Paraguay and Argentina (Sergio *et al.,* 1997; Broggi and Molto, 2001). The fungus has been isolated from rice seeds and maize seeds in Egypt (Madbouly *et al.,* 2012). The fungus has been reported to produce fumonisins, moniliformin, trichothecenes (T2) and zearalenone (Desjardins, 2006; Zaccardeli *et al.,* 2006; Lezar and Barros, 2010). There are risks of contamination of rice grains with mycotoxins of F. semitectum in the stores of South Africa. This is the first report of isolation of F. semitectum from rice in South Africa.

Other Fungi

Apart from Fusarium species, other fungi such as Alternaria alternata, Alternaria longipes, Cochliobolus miyabeanus, Nigrospora sphaerica, Phoma eupyrena, Phoma jolyana, Phoma sorghina and Pithomyces species were found in association with diseased rice plants and rice seeds in South Africa. The findings and conclusions of these other fungi are summarized, discussed and presented hereunder as follows:

Alternaria alternata and A. longipes

Alternaria alternata was isolated from diseased rice plants showing characteristicsymptoms of numerous minute dark brown or black spots on the leaves of rice plants in the fields of warm regions in the North West and Northern Cape Provinces.The fungus was also isolated from a rice seed sample from the warm region in the Mpumalanga Province of South Africa. Alternaria alternata has been reported to cause minute leaf spot of rice in Turkey (Copcu and Karaca, 1983) and glume spotting of rice in Malawi (Siddiqi, 1980). The fungus has also been reported as a seed – borne pathogen of rice (Moubasher *et al.,* 1972; Koroleva *et al.,* 1984; Ou, 1985; Saini, 1985; Lee *et al.,* 1986; Broggi and Molto, 2001; Butt *et al.,* 2011). Alternaria alternata is not just a weak parasite, but was found as pathogen of several crop plants in various countries of the world. The fungus has been reported to produce various mycotoxins such as fumonisins (Fb1, FB2. FB3), a family of food borne carcinogenic mycotoxins in culture (Chen *et al.,,* 1992; Abbas and Riley, 1986; Mirocha *et al.,* 1996; Rheeder *et al.,* 2005). Production of other mycotoxins such as alternariol, alternariol, monomethyl ether, alternuene and tenuazonic acid have also been reported (Meronuck *et al.,* 1972; Petro *et al.,* 1973; Wei and Swartz, 1985; Pose *et al.,* 2004). It has been reported that A. alternata might be one of the etiological factors for human esophageal cancer in Linxian, China (Dong *et al.,* 1987; Trucksess and

Pohland, 2001). Alternaria toxins have been demonstrated to be produced by Alternaria species on wheat (Magan and Lacey, 1985) and on sorghum (Sauer *et al.,* 1978; Magan and Baxter, 1984). Therefore, there are risks of contamination of rice and other cereal grains with mycotoxins in the stores of South Africa. This is the first part of isolation of A. alternata from rice in South Africa. In this investigation, A. longipes was isolated from a rice seed sample from the warm region in the Mpumalanga Province of South Africa. The fungus has been reported to produce mycotoxins such as alternariol, alternariol monomethyl ether and tenuazonic acid (Pose *et al.,* 2004). This is the first report of isolation of a longipes from rice in South Africa. The fungus has not been reported from rice before from any country in the world.

Cochliobolus miyabeanus and N. sphaerica

Cochliobolus miyabeanus was isolated from diseased rice plants showing symptoms of dark brown spots on both leaf surfaces in the fields of the North West and Northern Cape Provinces, during this investigation. The brown spot symptoms were found on leaves of both young and adult plants. It was also isolated from a rice seed sample from Mpumalanga Province. The fungus is well known as causal pathogen of brown spot of rice in various countries of the world (Padmanabhan, 1973; Webster and Gunnell, 1992; Agrios, 2005). The fungus has been isolated from the sheath of rice in India (Singh *et al.,* 2005). The brown spot of rice caused by C. miyabeanus has also been reported before from rice in South Africa (Gorter, 1977). In this investigation, N. sphaerica has been isolated from a rice seed sample from the Mpumalanga Province, South Africa. This is the first report of the isolation of N. sphaerica from rice in South Africa.

Phoma eupyrena, P. jolyana and P. sorghina

Phoma eupyrena has been isolated from a rice seed sample from Mpumalanga Province of South Africaduring this study. Phoma eupyrena is known as secondary unharmful organisms on potato tuber (Malcolmson, 1958; Dorenbosch, 1970). There is no pathogenicity report of P. eupyrena. This is the first report of P. eupyrena from rice in South Africa. On the other hand, P. eupyrena has not been reported from rice before from any other country in the world. In this study, P. jolyana has been isolated from a rice seed sample from the Mpumalanga Province. The fungus has been reported from different kinds of plants including rice (Boerema *et al.,* 1971) from different regions of the world.

This is the first report of P, jolyana from rice in South Africa. Phoma sorghina has been isolated from diseased rice plants showing sheath rot symptoms in the fields of North West and Northern Cape Provinces of South Africa. The fungus was also isolated from a rice seed sample f4rom Mpumalanga Province, South Africa. The fungus has been reported as a pathogen of sheath rot of rice in India (Ram *et al.,* 2005). Phoma sorghina was reported as the causal agent of glume blight of rice in South Africa (Gorter, 1977). Phoma sorghina has been reported from rice seeds in Brazil (Malavolta *et al.,* 2007), from sorghum grains originating from South Africa and Texas, USA and from pearl millets in Namibia (Pazoutova, 2009).

Pithomyces species

In this study, Pithomyces species have been isolated from a rice seed sample from Mpumalanga Province of South Africa. Pithomyces chartarum has been isolated from rice seeds in Cuba (Hilda *et al.,* 2003). The fungus has been reported to cause leaf damage to wheat in Europe (Toth *et al.,* 2007). This is the first report of Pithomyces species from rice in South Africa. Various mycoflora have been isolated from diseased rice plants and rice seeds in South Africa. There are risks of infection of rice grains with various fungi resulting in contamination of rice grains with multiple mycotoxins produced particularly by the species of Fusarium and Alternaria in the fields as pre – harvest and in the stores as post - harvest in South Africa. Mycoflora have negative impacts on plant health and human and animal health (Marasas *et al.,* 1984; Peraica *et al.,* 1999; Desjardins *et al.,* 2000; Desjardins, 2006; Van Rensburg, 2012; Latiffah *et al.,,* 2013). The co – occurrence of Mycoflora, aflatoxins and fumonisins with high concentrations in rice and maize seeds have been reported from markets of various districts in Cairo, Egypt (Madbouly *et al.,* 2012). Total aflatoxins and fumonisins in rice averaged 5.15 and 1014ug/kg respectively. Total aflatoxins and fumonisins were detected in maize averaged p, 75 and 33ug /kg respectively.

Conclusion

Accurate identification of fungi such as Fusarium species and fungi other than Fusarium spp in association with rice plants and rice seeds in South Africa is important because, they cause diseases in plants and produce mycotoxins and cause mycotoxicosis in humans as well as animals. It is therefore most important to obtain information on the Mycoflora associated with rice plants and rice seeds in South Africa, their phytopathogenecity, control and other variables for the management of effective disease control processes and purposes. In many ways this study is landmark research because, the growing of rice in South Africa is at a very rudimentary stage of development and, there is thus a paucity of research initiatives in this direction. The research is important from the perspective of developing a data base on rice research in South Africa as this facet of agriculture is enhanced and further developed. This study has contributed significantly to the identification of diseases hitherto not identified in rice and rice seeds within South Africa and, other parts of the world. More research in this direction is therefore, urgently required in South Africa. The study showed the impact of mycoflora associated with Oryza Sativa (Rice) in South Africa and documented appropriately some important issues that emanated from the research undertaken. It is hoped that this study will assist scientists and researchers involved in rice research to evaluate the findings of this study, in order to further enhance and consolidate their own research initiatives, and enhance further research in this direction in South Africa.

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