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Seed-Borne Fungi Associated with Rice Seeds Varieties in Bongor, Chad Republic

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A B S T R A C T

Fungi associated with seeds of eight varieties of rice collected from Bongor locality in Chad, were detected using blotter paper and agar plate methods. The fungi were isolated from rice seeds and identified based on their typical structure and basic characteristics. Twelve fungi species were isolated and identified with blotter paper methods while twenty three were identified using agar plate method. These findings revealed the presence of diverse fungi species associated with rice seeds varieties. Further research on the economic importance of these seed borne fungi with regards to seed germination and seedling infection resulting from these fungi is thus recommended. Furthermore, this study, although considered as preliminary, suggests that the fungi detected could be potential threat to rice production and a source of reflection of the possible diseases that could interfere with rice cultivation in Chad.

Introduction

Rice (*Oryzae sativa* L.) belongs to family *Poaceae* with two domesticated species of genus *Oryzae*. *Oryzae sativa* originates from tropical and subtropical southern Asia, while the African rice, *Oryzae glaberrima*, originates from West Africa (Renato *et al*., 2008; Habib *et al*., 2012). The crop is one of the most important food crops in the world which forms the staple diet of about 2.7 billons people and has become a commodity of strategic significance across many African countries (ICAR, 2006). It is also considered as the second most important cereal in the world, which provides about 95 % of food requirements of the world population (Juliano, 1993; Boumas, 1985). Produced in approximately 110 countries, including in variable degrees, in all west African countries, rice forms a major part of most people's diet in Africa after maize, sorghum, millet and to an extend in most developing parts of the world (Purseglove, 1988; Sakariyawo *et al*., 2013). In Chad, rice is grown either under upland or lowland conditions, with or without irrigation, for its seeds rich in starch, and is consumed as seeds, meal or in soups (Ngakou *et al*., 2013). As in most of African countries, rice has entered in the eating habits of Chadians for the past several years. It is therefore

grown as a cash crop or is consumed locally in rural areas in the south (USAID, 2007). Its production is estimated at about 378.246t (ONDR, 2015). But the quantities produced are not enough to meet the national demand and the country continues to rely on importing rice from Asian countries. The low productivity is attributed to a number of important factors, which are seed-borne diseases, causing up to 50-80% yield losses depending on the crop susceptibility, disease severity and agro-ecological factors (Raymundo, 1980).

Seed borne diseases may not only introduce new pathogens or affect quantitatively and qualitatively the crop yield, but also contaminate permanently the soil (Anselme, 1981). Many of these diseases are of fungal origin (Nsemwa and Wolfhechel, 1999), transmitted mainly through use of infected seeds for planting. Studies carried out by (Biruma *et al*., 2003; Kanobe *et al*., 2004) showed a wide range of seed borne fungi pathogens on the farmers saved rice seeds, which they mainly relay on to propagate the next generation of crop. Most seed borne diseases caused by the fungi pathogens are disastrous as they may decrease seed germination, cause seed discolouration; produce toxins that may be injurious to man and domestic animals. Several seed borne fungi associated with rice seeds have been isolated in many countries including Nigeria (Suleiman and Omafe, 2013); Pakistan (Butt *et al*., 2011); Egypt (Madbouly *et al*., 2014), Bangladesh (Ora *et al*., 2011) and Cameroon (Nguefack, 2007). But to the best of our knowledge, no report is available on seed borne fungi status of rice in Chad. Increasing rice production and subsequent reduction of its international importation and endemic plant pathogens continue to be a challenge in safeguarding plant health in Chad. Therefore, early and accurate diagnoses and pathogen surveillance will give time for development and application of mitigation strategies. So, the assessment of seed health standard of rice is very important for farmers and food security as it is a first line approach in managing seed borne diseases of plants. Furthermore, the quality of planted seeds has a critical influence on the ability of crops to become established and to realize their full yield potential and value (McGee, 1995). The present study was carried out to detect and identify the fungi associated with rice seeds varieties from Bongor locality in Chad.

Materials and Methods

The experiment was carried out at the Laboratory of Phytopathology at the University of Dschang, Cameroun. Eight rice varieties were collected from Bongor rice Operating Station, Chad in December 2014. The samples were enclosed in paper bags with proper labelings, taken to the laboratory and kept in the refrigerator at 4 ± 1°C until used. Detailed information on varieties used is given in Table 1.

Detection of fungi from seeds

Rice seed varieties were assayed for the presence of fungi by the Standard Blotter Methods and Agar plate Methods following the rules of International Seed Testing Association (ISTA, 2001)

Blotter paper methods

Seeds were surface sterilised using sodium hypochlorite ($NaOCl_2$) diluted at 2% for 5 minutes. Four hundred seeds were used for each variety maintaining sixteen replications. Twenty five seeds were placed in three layers on moist blotting paper in each Petri dish. The Petri dishes were incubated at 24 ± 2°C under alternating cycles of 12 hrs darkness and 12 hrs light provided by white fluorescent tube (Mazda,

T8 36W). Data regarding germination and infection were recorded 7 days after the incubation period and the results were recorded in percentages. Each of the incubated seeds was then examined for fungi growth under a stereo-microscope (20X). In fewer cases the fungi from the incubated seeds were transferred to PDA medium in Petri dishes aseptically and incubated under controlled temperature (20 ± 2°C) for 3-7 days and then examined. Most of the associated pathogens were detected by observing their growth characteristics on the incubated seeds on Blotter paper following the keys outlined by Champion (1997), Mathur and Kongsdal (2003) and Warharm *et al.*, (2008). Identification was also supplemented by microscopic examination of spores and fruiting bodies using a compound microscope and relevant books. The frequency of each fungus was determined from the percentage of the colonies of all the fungi developed as followed; F = NF / NT × 100, where NF is the number of specific identified fungus and NT, the total number of detected fungi.

Agar plate method

In this method, two hundreds seeds were tested for each variety maintaining twenty replications. Surface sterilised seeds as above were plated (10 seeds/Petri dishes) on the potato dextrose agar (PDA) medium prepared from 250 g of Irish potatoes, 20 g of dextrose, and 20 g of agar-agar and 1000 ml of distilled water. The plated seeds were incubated for 7 days at 20 ± 2°C in darkness. At the end of the incubation period, fungi developed from the seeds on the agar medium were examined and identified based on colony characteristics and morphology of fruiting bodies under a compound microscope. In the method, more than one type of fungi colonies were produced, and the identification was done on the most frequently occurring colony present in the Petri dishes and then the second most frequent and so on. Thereafter, the identification of different colonies was done visually and then under a stereomicroscope followed by an examination of the fruiting bodies under a compound microscope. Once the identification was done, the frequency of each fungus was determined as above.

Design of the Experiment: The experiment was conducted following Completely Randomized Design (CRD) with sixteen replications. The recorded data on percentage germination and fungi frequency were statistically analyzed using SPSS statistical package version 17.

Results and Discussion

All the varieties tested were found to be infected by fungi but at different levels.

Using the Blotter method, the highest frequency of 11.7 - 19.8%, was recorded with. *Alternaria padwickii*, followed by *Curvilaria lunata*, (5.9-14%) *Fusarium oxysporium* (9.9-13.5%) and *Verticillium sp* (2-9.5%). However, their frequency of occurrence varied from one variety to another. *Fusarium culmorum* was present in varieties D3, TOX and WITA9 at low frequency of 2.4-4.6% while *Melanospora zamiae* was found only in D1 variety (3.8%) (Table 3). The germination percentage ranges from 87% to 100% with D1 recording the least and D4 and TOX the highest germination percentage. Infection percentage ranges from 14% to 23% for D1 and WITA9 and DA, respectively. Variety D4 with the highest germination percentage (100%) recorded the highest infection percentage of 23% (Table 2).

In Agar plate method, among the fungi detected, ten were frequently isolated in

seeds of the eight varieties of rice. These were *Alternaria padwickii*(8.5-17.5%), *Aspergillus flavus* (2.7-9.5%), *Aspergillus niger* (1.5-7.7%), *Curvularia lunata* (5.1-10.1%), *Curvularia oryzae*(1.7-7.3%), *Fusarium oxysporium* (5.6-13.6%), *Rhizoctonia solani* (5.5-7.8%), *Rhizopus stolonifer* (0.2-7.7%), *Tilletia caries* (0.8-7.2%) *and Verticilliumsp* (3.6-6.9%). The other species; *Alternaria brassicae, Aspergillus Niger, Cladosporium sphaerospermum, C. pallescens, Epicoccum nigrum, Fusarium avenaceum, Fusarium culmoreum, Microdichium oryzae, Penicillium sp, Phoma lingan, Rhizopus stolonifer, Tilletia caries* were found in low frequency.

The present study was focused on the survey of seed borne fungi associated with rice varieties form Bongor locality, Chad. Twelve fungal species were isolated and identified with blotter method while twenty were obtained by agar plate method. These results were in coherence with those of many research groups around the world (Imolehin, 1987; Khan *et al*., 1988, 2000; Kim and Lee, 1989; Odebunmi, 1989; Wahid *et al*., 1993; Javaid *et al*., 2002, Ibiam *et al*., 2006). Many of the isolated fungi have been reported to be associated with seeds of other crops (Tsopmbeng, 1994; Tsopmbeng and Fomengia, 2015). Some of them are also known to cause seed rot, decrease seed germination and cause pre and post damping off and seedling death (Al-kassam and Monawar, 2000).

Alternaria padwickii, Curvilaria lunata, Fusarium oxysporium, were found in high frequencies. This high frequency has been reported in previous studies on the seeds of rice in India (Archana and Prakash, 2013) and Pakistan (Khan, 2000; Javaid *et al*., 2002). Earlier observations reported the occurrence of *Pyricularia oryzae, Alternaria alternata, A. padwickii, A. longissima, Curvularia oryzae, C. lunata, Drechslera oryzae, A. niger, Fusarium moniliforme, F. semitectum, F. oxysporum, F. solani*and species of *Phoma, Cercospora, Chaetomium, Sclerotium, Penicillium, Myrothecium* and *Colletotrichum* from seeds of rice varieties (Khan, 2000; Wahid *et al*., 2001; Javaid *et al*., 2002; Nguefack *et al*., 2007). In this study, *Pyricularia oryzae,* known for a long time as rice seed borne fungus (Manandadhar *et al*., 1998) was not isolated from any of the rice varieties, confirming the statement by Lamrani *et al.,* (2013) who found that this fungus was less common on seeds. Aluko (1969) tested rice seed samples for seed borne fungi and indicated the scarcity of this fungus in Nigeria. Its absence in seed of rice varieties in Chad may indicate that blast caused by *Pyricularia oryzae* will be less frequent on rice grown in the field.

Alternaria padwickii was consistently isolated from rice seeds. According to Butt *et al.,* (2011); Khan *et al.,* (2000); Bhutta and Hussain (1998) and Khan *et al.,* (1988), this fungus has a pathogenic role in rice seeds**.** Similarly Utobo *et al*., (2011) isolated *Alternaria padwickii* from stored rice grains and found that it was associated with high frequency.

According to Lamrani *et al.,* (2013), *Alternaria Padwickii* colonizes a variety of seeds thus reducing the percentage germination and seed rot. Species of the genus *Curvularia*, in particular *C. lunata* has been reported to infect the embryo of the seeds therefore reducing the percentage germination of rice seeds (Imolehin, 1983; Bautista and Opina, 1991). Butt *et al.,* (2011), Utobo *et al.,* (2011) and Khanum and Khanzada (1989) reported *Curvularia lunata* in different rice varieties and labelled it as seed-borne.

Table.1 Characteristics of different rice varieties

Variety	Cycle (days)	Production system	Mean yield (t/ha)	Origin	Shape of the grains
TOX	90	Rain fed and irrigated lowland	5	Imported	Long
WITA9	120	Rain fed and irrigated lowland	10	Imported	Long and thin
CH8	120	Rain fed and irrigated lowland	6	Imported	Long
CH3	120	Rain fed and irrigated lowland	5	Imported	Short and thin
D6	120	Rain fed and irrigated lowland	5	Local	Long
D4	120	Rain fed and irrigated lowland	5	Local	Long
D3	120	Rain fed and irrigated lowland	5	Local	Long
D1	120	Rain fed and irrigated lowland	5	Local	Long

Table.2 Percentage seed germination and seed-borne fungal infections of eight rice varieties collected from Bongor locality.

Variety	Seed germination	Seed-borne fungi infections
CH3	$97\pm2^{a*}$	16 ± 2^{ab}
CH8	90 ± 2^{b}	18 ± 2^{ab}
D1	87 ± 2^{b}	14 ± 2^{b}
D3	96 ± 2^{a}	22 ± 2^{a}
D4	100 ± 2^{a}	23 ± 2^{a}
D6	100 ± 2^{a}	16 ± 2^{ab}
TOX	$96,6\pm2^{a}$	18 ± 2^{ab}
WITA9	96 ± 2^{a}	14 ± 2^{b}

*Means followed by the same letter(s) in each column are not significantly different by at 5% probability level using DMRT (Duncan Multiple Range Test). values were based on 400 seeds per variety.

Table.3 Percentage frequency of different fungi isolated on rice varieties by blotter method

Fungi isolated	Rice seed varities							
	CH3	CH8	D1	D3	D4	D6	TOX	WITA9
Alternaria alternata (Fr.) Keissl.	$2,9^{de*}$	$5,8^{d}$	$2,7^{d}$	0^{e}	0^{f}	$4,6^{d}$	$5,9^{c}$	$2,9^{c}$
Alternaria padwickii (Ganguly) M. B. Ellis	$16,6^{a}$	$15,8^{a}$	$18,8^{a}$	$11,7^{ab}$	$17,8^{a}$	$17,4^{a}$	$16,9^{a}$	$19,8^{a}$
*Aspergillus flavus*Link.	0^{e}	0^{e}	$2,7^{d}$	$7,5^{bc}$	$5,3^{cd}$	$4,2^{d}$	$4,7^{d}$	0^{e}
Bipolaris hawaiiensis (M.B.Ellis)	$4,5^{d}$	$7,2^{c}$	$3,7^{d}$	$8,4^{b}$	$6,6^{c}$	0^{e}	$6,5^{c}$	$2,9^{de}$
Bipolaris oryzae (Breda de Haan) Shoem.	$7,5^{c}$	$11,6^{b}$	$8,9^{bc}$	$9,6^{b}$	$10,5^{b}$	0^{e}	0^{de}	$12,3^{b}$
Curvilaria lunata (Wakker) Boedijn	$7,5^{c}$	$5,9^{d}$	$7,7^{c}$	$11,7^{b}$	14^{ab}	$12,4^{b}$	$7,6^{c}$	$11,7^{bc}$
Curvilaria oryzae Bugnicourt	$4,4^{d}$	0^{e}	$9,5^{b}$	0^{e}	0^{f}	$13,6^{b}$	$13,8^{b}$	0^{e}
Fusarium culmorum (W.G. Sm.) Sace.	0^{e}	0^{e}	0^{f}	$2,4^{d}$	0^{f}	0^{e}	$4,6^{cd}$	$2,5^{b}$
Fusarium oxysporium Schltdl.	$13,5^{a}$	$12,2^{b}$	$10,8^{b}$	$13,2^{a}$	$12,1^{b}$	$10,9^{c}$	$12,7^{bc}$	$9,9^{c}$
Melanospora zamiae Corda	0^{e}	0^{e}	$3,8^{d}$	0^{e}	0^{f}	0^{e}	0^{de}	0^{e}
Rhizoctonia solani Kühn	6^{bc}	$7,3^{c}$	$5,7^{c}$	$2,8^{d}$	0^{f}	$4,5^{d}$	$5,6^{c}$	$4,4^{d}$
Verticillium sp	$9,5^{b}$	$3,9^{bc}$	$1,7^{e}$	2^{d}	$2,1^{e}$	$4,6^{d}$	$3,3^{d}$	$2,6^{de}$

Values with different letters within a column differ significantly at 5% level of significance using DMRT (Duncan Multiple Range Test). Data present the mean of three replications.

Table.4 Percentage frequency fungi isolated on rice varieties by agar plate method

Fungi isolated	Rice seed varities							
	CH3	CH8	D1	D3	D4	D6	TOX	WITA9
Alternaria alternata (Fr.) Keissl.	5,1^{cd}	2,6^{e}	1,9^{g}	0^{h}	3,8^{e}	1,7^{f}	3,2^{f}	4,7^{f}
Alternaria longissima Deighton & MacGarvie	1,9^{d}	2,5^{e}	4,9^{d}	4,6^{d}	3,5^{e}	3,1^{d}	1,9^{d}	0^{j}
Alternaria padwickii (Ganguly) M. B. Ellis	11,7^{a}	17,5^{a}	8,1^{b}	15,4^{a}	12,5^{a}	9,5^{a}	13,2^{a}	13,6^{a}
Aspergillus flavus Link.	7,6^{c}	2,9^{e}	4,5^{d}	2,7^{f}	8,5^{c}	8,2^{ab}	9,5^{b}	7,8^{cd}
Aspergillus niger van Tiegh.	5,2^{cd}	2,8^{e}	3,8^{e}	1,5^{fg}	2,7^{f}	2,5^{e}	7,7^{c}	5,9^{e}
Bipolaris hawaiiensis (M.B.Ellis)	4,5^{d}	4,9^{d}	3^{ef}	3,6^{e}	0^{h}	1,3^{f}	5,2^{d}	0^{j}
Bipolaris oryzae (Breda de Haan) Shoem	5,1^{cd}	6,4^{c}	0^{h}	4,7^{d}	4,8^{de}	0^{g}	0^{i}	3,8^{g}
Bipolaris spicifera (Bainier) Subram.	4,7^{d}	6,6^{c}	0,9^{gh}	1,6^{de}	2,4^{f}	2,9^{de}	4,4^{e}	0^{j}
Cercospora kikuchii (Mats. & Tom)	0^{f}	0^{f}	0^{h}	2,4^{f}	3,1^{e}	2,9^{de}	0^{i}	0^{j}
Cercospora oryzae	0^{f}	0^{f}	5,5^{d}	0^{h}	0^{h}	0^{g}	0^{i}	3,8^{g}
Curvilaria lunata (Wakker) Boedijn	8,1^{b}	5,1^{f}	2,8^{f}	7,2^{cd}	10,7^{b}	9,4^{a}	7,3^{c}	10,1^{b}
Curvilaria oryzae Bugnicourt	5,6^{cd}	6,3^{c}	7,3^{c}	9,3^{c}	7,3^{c}	1,7^{f}	4,8^{e}	2,7^{h}
Epicoccum purpurescens Ehrenb.	3,9^{d}	5,3^{b}	0^{h}	5,8^{d}	0^{h}	0^{g}	5,6^{d}	9,5^{c}
Fusarium culmorum (W.G.Sm.) Sace	0^{f}	0^{f}	6,2^{c}	1,7^{fg}	0^{h}	6,9^{bc}	2,5^{g}	4,7^{f}
Fusarium moniliforme J. Sheld.	0^{f}	0,4^{ef}	4,6^{d}	1,6^{fg}	0^{h}	0,7^{fg}	3,4^{f}	1,8^{c}
Fusarium oxysporium Schltdl.	5,6^{cd}	12,8^{b}	13,6^{a}	10,7^{b}	11,1^{b}	7,1^{b}	12,3^{a}	14,5^{a}
Geotrichum sp	0^{f}	1,4^{e}	1,3^{g}	0^{h}	0^{h}	1,5^{f}	1,1^{h}	0^{j}
Microdochium oryzae (Fr.)	0,9^{e}	0^{f}	0^{h}	0^{h}	0^{h}	0^{g}	2,2^{g}	0^{j}
Phoma sp. Westend.	0^{f}	0^{f}	0,7^{gh}	0^{h}	0^{h}	0^{g}	2,5^{g}	2,6^{h}
Rhizoctonia solani Kühn	5,5^{cd}	7,8^{c}	7,2^{c}	3,6^{e}	4,5^{de}	6,1^{c}	4,4^{e}	3,6^{g}
Rhizopus stolonifer Ehrenb.	7,7^{c}	1,7^{e}	3,9^{e}	0,2^{g}	1,6^{g}	3,9^{d}	5,6^{d}	6,4^{d}
Tilletia caries (DC.) Tulasne	7,2^{c}	5,9^{cd}	2,6^{f}	5,4^{d}	2,5^{f}	3,7^{d}	0,8^{hi}	1,1^{i}
Verticillium sp	4,9^{d}	5,2^{d}	3,6^{e}	3,6^{d}	4,4^{de}	6,9^{bc}	5,5^{d}	3,6^{g}

Values with different letters within a column differ significantly at 5% level of significance using DMRT (Duncan Multiple Range Test). Data present the mean of three replications.
Four hundred seeds were tested for each variety

According Duraiswamy and Mariappan (1980), the genus *Curvilaria*, is also involved in the discoloration of the rice seeds. As for *Fusarium* spp, they are major plant pathogens, and many are seedborne (Neergaard, 1979). *F. oxysporum*, an agent of *Fusarium* wilt of many hosts, was the most important species isolated. This fungus has a wide host range, including numerous *formae speciales*, some of which contain pathogenic races causing devastating wilt disease (Neergaard, 1979). Also, *F. oxysporium* has the ability to behave either as saprotrophe or parasite that attacks many plant species (Champion, 1997). Their presence in high frequency in rice seed varieties are not known and need to be investigated. In the present investigation, *Fusarium* spp, *Curvularia lunata* and *Bipolaris* spp were found more frequently

with agar plate method whereas *Alternaria padwickii*, *Fusarium oxysporium* were found with the blotter paper method. The study also revealed that some fungi showed high frequency with agar plate method whereas some others were more frequent in blotter paper method. Ashfaq *et al.,* (2015) evaluated the two methods and found that fungi like *Penicillium globosum*, *Rhizoctonia sp*., *Phoma sp.* were isolated in higher frequency from blotter paper method and *Curvularia lunata* and *Drechslera sp.* from agar plates. Khan *et al.,* (1988) had also reported the blotter paper method as the most suitable method for detecting *Alternaria alternata, A. tenuissima, Aspergillus niger, A. flavus, A. terreus, Chaetomium globosum* and *Curvularia lunata* on rice seeds; whereas the agar plate method showed higher percent frequencies of isolation of *Drechslera* spp. and *Curvularia* spp. However, Agarwal *et al.,* (1972) found out that the blotter paper method is a better method for the isolation of *Drechslera oryzae* and *Alternaria padwickii* than the agar plate method.

In conclusion, the present study reveals the presence of various fungi associated with rice seeds from Bongor locality in Chad and indicates the possibility of disease occurrence when such infected seeds are planted. Although the results of the present study may be considered preliminary, they suggest fungi associated with rice seeds are potential threat to its production. Research into the economic importance of these seed borne fungi with regards to seed germination and seedling infection resulting from these fungi is recommended for better seed health management.

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