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Seedborne Mycoflora Associated with Rice and Their Influence on Growth at Abakaliki, Southeast Agro-Ecology, Nigeria

[1]E.B. Utobo, [2]E.N. Ogbodo and [1]A.C. Nwogbaga

[1]Department of Crop Production and Landscape Management, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
[2]Department of Soil and Environmental Management, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

Abstract: Seed borne fungi associated with eight hybrid (H) and three local check (LC) rice varieties and their effects on grain germination and seedling vigour were studied during the 2007 and 2008 harvesting seasons. A total of 9 fungal genera were isolated and identified from the seed samples collected from four rice fields located at Nwaezenyi-Izzi, Enyigba-Abakaliki, Ishieke-Ebonyi and Amagu-Ikwo within Abakaliki agricultural area of Southeastern Nigeria. Most frequently isolated fungi was *Trichoconis padwickii* (37.14% H; 36.41% LC), followed by *Helminthosporium oryzae* (17.14% H; 16.85% LC), *Fusarium moniliforme* (14.29% H; 14.13% LC), for hybrid and local check rice varieties respectively. The highest and lowest frequencies of fungal occurrence were observed on grains of long ridge (24.00%) and CP (4.57%) for hybrid and IR-8 (58.15%) and Kpurukpuru (15.76%) for local check rice varieties respectively. Percentage of germination and seedling vigour were found significant ($p<0.05$) from hybrid to local check rice varieties. Maximum number of germinated seed at 5 (57.15%), 9 (68.30%) and 14 (70.30%) days after sowing (DAS) were recorded from seed samples of hybrid rice varieties and minimum number of germinated seeds at 5 (31.10%), 9 (51.20%) and 14 (55.72%) DAS were observed from that of local check rice varieties. Hybrid rice showed higher vigour in terms of germination (82.74%), root length (10.25 cm), root weight (0.21g), shoot length (6.27 cm), root weight (0.48 g) and vigor index (1161.36) when compared to local check rice varieties. Our findings necessitate the use hybrid rice grains for optimum germination, seedling vigour, growth and yield.

Key words: Rice % Seed borne fungi % Seed germination % Seedling vigour

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the main staple foods of man and is grown in almost all the tropical and subtropical regions of the world. Abakaliki agricultural area of Southeastern Nigeria is a major rice producing block in Nigeria. The area has the potential for achieving national self sufficiency in rice production. However the yield of the crop has continued to decline. This yield decline may be as a result of seed borne pathogens present externally or internally on the rice grains [1]. These seed borne pathogens according to Bateman and Kwasna [2] and Khanzada *et al*. [3] may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection. Moreover, Danquah and Mathur [4] reported that infected seeds are often discoloured, an indication of poor grain quality. Under epidemic conditions, total loss of a rice plantation to the pathogen may ensue [5].

Several fungal pathogens have been isolated from rice grains and have been reported to be responsible for a number of diseases right from the nursery to the field by Ibiam *et al*. [6]. Odebunmi - Osikanlu [7] isolated *Fusarium moniliforme, C. lunata. Helminthosporium oryzae, Rhynchosporium oryzae* from the six rice seed varieties (IRAT. 110, COL.38, C22, TOX494-SLR, DJII-509 and F.H. 109). Kim and Lee [8] reported that *Aspergillus terreus, A. ochraceus,* among other *Asperigllus spp* and

Corresponding Author: E.B. Utobo, Department of Crop Production and Landscape Management, Faculty of Agriculture and Natural Resources Management, Ebonyi State University, P.M.B. 053 Abakaliki, Nigeria. Tel: +234 8035434435.

6 *Penicillium spp* were isolated from deterioriated rice seed samples in Korea The fungal pathogens recorded by Imolehin [9] on twenty-two seed samples of rice cultivars from south-western Nigeria included the following: *Drechslera oryzae, Curvularia lunata, Fusarium moniliforme* and *Rhynchosporium oryzae,* while the saprophytic fungi were *Aspergillus spp, Penicillium spp, Rhizopus spp, Chaetomium spp, Trichoderma spp and Cladosporum spp*. He further stated that *Fusarium moniliforme* and *Drechslera oryzae* were the major pathogens that caused devastating seedling disease of rice in the field (*D oryzae* 12%, *F moniliforme* 40%). Seedborne mycoflora of rice reported by Khan *et al*. [10] included *Trichoconis padwickii*, *Helminthosporium oryzae, Curvularia lunata, Cochlobolus lunatus, Alternaria tenuis* and *Epicoccum* sp. Other seed borne fungi have been isolated from rice but have not been shown pathogenic on rice as a result of their presence on seed [5]. There is no adequate information, however, on the seedborne fungi of rice in Abakaliki agricultural area and their role in rice production in Ebonyi State. This study therefore was carried out to study the incidence of seedborne mycoflora of hybrid and local rice grains from various rice locations in Abakaliki relative to seed germination and seedling vigour.

MATERIALS AND METHODS

Isolation and Identification of Fungi: Eleven rice varieties, including eight hybrids (Faro-15, Faro-16, Faro-44, Faro-51, Faro-52, Long ridge, CP and Iron and three local checks (IR-8, BG-12 and Kpurukpuru) varieties were collected from four major rice fields located at Nwaezenyi-Izzi, Enyigba-Abakaliki, Ishieke-Ebonyi and Amagu-Ikwo Local Government Areas of Ebonyi State. The names of the varieties were as used by the breeders. The rice grains were collected from the rice fields at harvest (matured and dried) and each variety from each location packaged in a sealed envelope to avoid contamination by secondary inoculum. Envelopes containing the rice varieties were properly labeled, giving information about the variety, date and place of collection.

The standard blotter method [11] was used to determine the occurrence of seedborne mycoflora on rice seeds 15 days after harvest. Three pieces of filter paper were soaked in sterilized water and were placed at the bottom of a 9 cm well labeled plastic petri dishes. Twenty five (25) seeds of each rice variety selected at random from each envelop were placed and spaced in each plastic petri dish using a pair of forceps, making sure that seeds are placed equidistantly with 15 seeds on the outer ring, 9 seeds at the inner ring and 1 seed in the middle. Sixteen petri dish plates were used for each sample. The lids of each petri dish were held in place with gummy cellotape. The petri dishes containing seeds were incubated at 20 ± 2°C for 7 days under alternating cycles of light and darkness of 12 hours each, using daylight fluorescent tubes (Philips TL 40W/54) as the NUV light source.

After the 7-day incubation period, individual rice seeds were examined under microscope in order to record the incidence of different seed borne fungi. With flamed-sterilized transfer needles, fungal growths on the grains were aseptically mounted in lactophenol blue on slides and examined under the stereo-binocular microscope for fungal diagnostic characteristics. For proper identification of fungi, semi permanent slides were prepared from the fungal colony and observed under compound microscope. Pure cultures of isolated fungi were obtained through transfers on Potato Dextrose Agar (PDA) medium.

A list of morphological characters of taxonomic importance such as spore size, shape, septation, colour and their arrangement on the conidiophores, appearance of the spore masses, character of the mycelium, density of the colony were compiled for each fungus. Identification of the fungus was perfumed using all the characteristics observed and identification reference manuals of Booth [12], Barnett and Hunter [13] and Watanabe [14]. The frequency of fungal isolation was also recorded.

Germination and Seedling Vigour Tests: The germination test was carried out using in between paper method [11]. Two sheets of square blotter papers (AGF 725-230 × 265 mm) were wetted in distilled water, leaving an adequate margin. One-hundred seeds were sown on it evenly and rolled. For each treatment, 400 seeds were sown. The rolls were placed upright inside a plastic bag to avoid drying during incubation at 28°C, which alternated between 12 h NUV light and 12 h darkness using daylight fluorescent tubes (Philips TL 40W/54) as the NUV light source. Germination was recorded at 5, 9 and 14 days after sowing (DAS) and was expressed in percentage (%).

Seedling vigor test was conducted in sand [11]. Plastic trays (18 × 9) were used. One hundred seeds were selected at random and sown on sand in each plastic tray in four lines (25seeds/line). A total of 400 seeds were

tested for each sample. Percentage germination was counted at 14 DAS. After 20 days, shoot and root length were measured. Fifteen seedlings from each tray were randomly selected for measurement of shoot or root length. The seedling vigour was determined following the formula of Baki and Andersen [15] as shown below:

Vigour index = (Mean of root length + Mean of shoot length) × Percentage of seed germination

Data collected were subjected to statistical analysis of variance (combined for the two years) to test for the significance of treatment effects using GenStat 5.0 [16].

RESULTS

A total of 9 fungal genera were found to be associated with the seed samples collected from the various field locations (Table 1). The associated fungi were *Rhizopus oryzae*, *Trichoconis padwickii*, *Helminthosporium oryzae*, *Fusarium moniliforme*, *Aspergillus niger*, *Curvularia lunata*, *Penicillium* sp., *Alternaria oryzae* and *Pyricularia oryzae*. They most belong to the class Dueteromycetes except *Rhizopus oryzae* that belongs to the class zygomycetes.

Frequency of isolation of each fungus is presented in Table 2. *Trichoconis padwickii* (37.14%) was the most predominant seed borne fungus isolated, followed by *Helminthosporium oryzae* (17.14%), *Fusarium moniliforme* (14.29%), *Aspergillus niger* (9.71%) for hybrid varieties and *Trichoconis padwickii* (36.41%), *Helminthosporium oryzae* (16.85%), *Fusarium moniliforme* (14.13%) and *Rhizopus oryzae* (10.89%) for local checks. *Trichoconis padwickii* (36.77%) was the most frequently isolated seedborne fungus irrespective of the source of the rice varieties tested in the study. This is followed by *Helminthosporium oryzae* (16.99%), *Fusarium moniliforme* (14.21%) and *Rhizopus oryzae* (8.64%). Two fungi namely *Alternaria oryzae* and *Pyricularia oryzae* were infrequently isolated in all. The rice varieties mostly affected was long ridge (24.00%) for hybrid and IR-8 (58.15%) for local checks respectively.

Germination at 5, 9 and 14 DAS were determined and the result presented in Table 3. Maximum number of germinated grain (57.15%) at 5 DAS was recorded from grain sample of hybrid rice and minimum number (31.10%) was from that of local checks. Maximum number of germinated grain (68.30%), 9 DAS was recorded from grains of hybrid rice and minimum count (55.73%) was from local checks. Maximum number of germinated grain (70.30%) at 14 DAS was recorded from grains of hybrid rice and minimum count (51.20%) was recorded from grains of local checks. Number of germinated grains increased from 57.15% to 70.30% for hybrid rice and 31.10% to 55.72% for local checks from 5 to 14 DAS respectively. Germination percentage of different duration (5, 9 and 14 DAS) were found significant ($p<0.05$) from hybrid to local rice checks.

In the seedling vigour experiment, hybrid and local rice grains was found to differ significantly ($p<0.05$) in all the parameters tested (Table 3). Highest germination (82.74%) was found in grain sample of hybrid rice and the lowest (68.36%) germination was observed in grains of local rice varieties. The root length ranged from 10.25 cm to 6.42 cm. The highest value (10.25 cm) was recorded in seedling of hybrid rice grain and the lowest (6.42 cm) was found in seedling of local rice checks. Highest root weight of 0.21g was found in seedling of hybrid rice grain and the lowest root weight of 0.10 g in seedling of local rice varieties. The highest shoot

Table 1: Associate fungi with grains of various rice varieties

		Rice Varieties										
		Hybrid								Local checks		
S/No.	Fungi Isolated	Faro-15	Faro-16	Faro-44	Faro-51	Faro-52	Long Ridge	CP	Iron	IR-8	BG-12	Kpurukpuru
A	Zygomycotina											
1	*Rhizopus oryzae* Went abd Gerlings	-	+	+	-	+	+	-	-	+	+	+
B	Deuteromycotina											
1	*Trichoconis padwickii* Ganguly	+	+	+	+	+	+	+	+	+	+	+
2	*Helminthosporium oryzae* Link exfr	+	+	+	-	+	+	-	+	+	+	-
3	*Fusarium moniliforme* (Schl.) Syn and Hans	+	+	+	-	+	+	-	-	+	+	+
4	*Aspergillus niger* Van Tiegham	+	+	+	-	+	+	-	+	+	-	-
5	*Curvularia lunata* (Walker) Boedjin	-	+	+	-	-	+	-	-	+	-	+
6	*Penicillium* sp.	-	+	-	-	+	-	+	+	+	-	-
7	*Alternaria oryzae* Nees ex. wallr	-	-	-	+	-	-	-	+	-	-	-
8	*Pyricularia oryzae* Cavara	-	-	+	-	-	-	-	-	+	+	-

Table 2: Frequency occurrence of associated fungi with grains of some rice varieties

S/No	Varieties	Trichoconis padwickii	Helminthosporium oryzae	Fusarium moniliforme	Aspergillus niger	Rhizopus oryzae	Curvularia lunata	Penicillium sp.	Alternaria oryzae	Pyricularia oryzae	Total	% Frequency
Hybrids (H)												
1	Long Ridge	23	11	4	-	2	2	-	-	-	42	24.00
2	Faro-16	7	4	8	-	6	5	4	-	-	34	19.43
3	*Iron*	4	3	-	3	-	-	4	5	-	19	10.86
4	Faro-52	3	-	10	7	1	-	1	-	-	22	12.57
5	Faro-44	9	4	1	2	2	3	-	-	1	22	12.57
6	Faro-51	11	3	-	-	-	-	-	1	-	15	8.57
7	Faro-15	6	5	2	-	-	-	-	-	-	13	7.43
8	CP	2	-	-	5	-	-	1	-	-	08	4.57
	Total	65	30	25	17	11	10	10	06	01	175	
	% Frequency	37.14	17.14	14.29	9.71	6.29	5.71	5.71	3.43	0.57		
Local Checks (LC)												
1	IR-8	34	15	8	12	15	9	11	2	1	107	58.15
2	BG-12	21	9	13	-	4	-	-	-	1	48	26.09
3	*Kpurukpuru*	12	7	5	1	1	3	-	-	-	29	15.76
	Total	67	31	26	13	20	12	11	02	02	184	
	% Frequency	36.41	16.85	14.13	7.07	10.89	6.52	5.98	1.09	1.09		
All Varieties												
	Total	132	61	51	30	31	22	21	08	03	359	
	% Frequency	36.77	16.99	14.21	8.36	8.64	6.13	5.85	2.23	0.84		

[a]Fungi isolated from seeds examined were not mutually exclusive

Table 3: Germination and seedling vigour of hybrid and local rice varieties collected from various farm locations in Abakaliki

	% Germination[a]			Seedling Vigour[b]					
Rice Samples	5 days	9 days	14 days	Germination (%)	Root length (cm)	Root weight (g|)	Shoot length (cm)	Shoot weight (g)	Vigour index (VI)
Hybrid	57.15a	68.30a	70.30a	82.74a	10.25a	0.21a	6.27a	0.48a	1161.36a
Local checks	31.10b	51.20b	55.72b	68.36b	6.42b	0.10b	3.72b	0.31b	565.00b
$LSD_{(0.05)}$	6.43	7.58	6.47	8.68	2.44	0.05	1.57	0.13	342.73

[a]Five samples (4 × 100 seeds/sample in 4 trays) were tested of each treatment

[b]Five samples (40 seedlings/sample) were tested of each treatment

length result (6.27 cm) was observed in seedling of hybrid rice grain and the smallest length (3.72 cm) was found in seedling of local rice checks. Shoot weight ranged from 0.48 g to 0.31 g, where the highest (0.48 g) root weight was found in seedling of hybrid rice grain and the lowest (0.31 g) was observed in seedling of local checks. Vigor index (VI) ranged from 1161.36 to 565.00. The highest VI of 1161.36 was found in seedling of hybrid rice grain and the lowest VI of 565.00 was recorded in seedling of local rice varieties.

DISCUSSION

Trichoconis padwickii was the most frequently isolated seedborne fungus irrespective of the source of the rice varieties tested in the study. *Trichoconis padwickii* poccurrence has been reported in Adani rice growing area of present Enugu State, Southeastern Nigeria [17] as in most other rice producing countries [18]. This pathogen causes loss in germination, viability and vigour of the rice grains [19]. It is found to be responsible for necrotic spots developed in the roots and coleoptiles of seedling. Heavily infected seedlings eventually die [20]. The second most isolated fungus is *Helminthosporium oryzae*. This pathogen has been shown to reduce rice yield in Nigeria, as in most other rice producing countries [21]. The pathogen has been reported to reduce seed germination [22, 23] and has also been shown to cause seedling blight [1]. The high frequency of isolation of *Helminthosporium oryzae* from the four locations of the rice fields may explain in part the high incidence of brown leaf spot in the Abakaliki agricultural area, Southeastern Nigeria. This pathogen may pose a serious threat to the rice industry in the area if adequate control measures are not implemented. This finding is in agreement with the work of Imolehin [5].

Furthermore *Fusarium moniliforme* is the third most frequently isolated fungus from grains in the study. Bedi and Dhaliwal [24] reported that infected seedlings often fail to emerge in cold, wet soil. This pathogen may therefore contribute in part to poor seed germination and reduced rice stands in many rice fields where infected seeds have been planted. *Fusarium moniliforme* is also known to cause discolouration of rice seeds [25]. Its frequent isolation from seeds indicates that it may contribute to poor grain quality of Ebonyi processed rice.

Aspergillus niger and *Rhizopus oryzae* although regarded as surface contaminates, were also frequently observed on rice grain samples. Besides the fact that some *Aspergillus* sp. produces aflatoxins, they have also been shown to deteriorate stored grains [26]. The work by Imolehin [5] has also associated these fungi with reduced germination of grain in the laboratory. He stated that these fungi, like rice pathogens, are therefore of economic importance in Nigerian rice production.

Pyricularia oryzae, a very important seedborne pathogen of rice [18, 21, 27], was only isolated from the rice cultivar, Faro 44 for hybrid varieties and, IR-8 and BG-12 for local checks respectively. Aluko [19] reported that 8 1% of the rice grain samples tested was infected by *Helminthosporium oryzae* compared with 8.4% infection by *Pyricularia oryzae* on rice seeds in Nigeria. This may indicate that blast caused by *Pyricularia oryzae* will be less frequent on rice raised on newly cultivated land compared with the brown spot diseases caused by *Helminthosporium oryzae.* Other seedborne fungi were rarely isolated from the rice seeds tested. Imolehin [5] reported that this does not indicate that they cannot cause serious diseases of rice under more favourable environmental conditions. For examples, *Curvularia lunata* was reported to cause glume and kernel discolouration on rice in Nigeria.

Germination and seedling vigour of rice grain samples collected from hybrid and local rice varieties showed significant variation. The grain samples of hybrid rice not only gave maximum germination but also yielded maximum number of seedling vigour. From this study it had been found that grains of local rice varieties resulted in poor germination and poor seedling vigour, when compared with the hybrid ones. These may be attributed to improved genetic make-up of the hybrid rice varieties. Farmers are therefore advised to use hybrid rice grains for optimum germination, seedling vigour, growth and yield.

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