

Survey on seed borne fungi and its effects on grain quality of common rice cultivars in the Mekong Delta

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ABSTRACT

Surveys on effect of discolored grains to grain quality of rice cultivars grown in some cultivated areas of the Mekong Delta have been implemented since 1997. Results indicated that there were nine fungal species found on 60 samples of 12 cultivars (improved and traditional) in Long An province by "Blotter method." *Curvularia* spp. (13.4%) is the most popular one, next to *Alternaria padwickii* (12.0%), *Bipolaris oryzae* (4.9%), *Sarocladium oryzae* (1.9%), *Fusarium gramineum* (1.5%), *Tilletia barclayana* (0.16%), *Phoma sorghina* (0.1%), *Cephalosporium oryzae* (0.34%), *Ustilagoideae virens* (0.05%). In Can Tho province, among rice samples of two cultivars collected from 10 farmers, eight fungal species were determined as *Alternaria padwickii*, *Bipolaris oryzae*, *Fusarium moniliforme*, *Fusarium pallidoroseum*, *Fusarium subglutinans*, *Microdochium oryzae*, *Nigrospora oryzae*, *Phoma* sp. and *Sarocladium oryzae*. Not much different seed pathogens in seed samples collected of two locations were recognized, but a little difference on detection frequency. Infection level of fungi was found in improved cultivars rather than landraces' seed samples. Effects of discolored grains to grain quality were also investigated, rice milling recovery and cooking and eating quality were significantly different from healthy seeds. Seed germination (%), seedling height (cm) were much affected by discoloration. In yield components, 1000-grain weight (g) was found to be mostly affected. However, there was no yield loss due to seed borne fungi.

Key words: Seed borne fungi, Discolored grain, Grain quality, *Alternaria padwickii*, *Bipolaris oryzae*, *Cephalosporium oryzae*, *Curvularia oryzae*, *Fusarium gramineum*, *Phoma sorghina*, *Sarocladium oryzae*, *Tilletia barclayana*, *Ustilagoideae virens*.

INTRODUCTION

Rice is the most important cereal crop in Vietnam. In recent years, Vietnam has become a major rice exporter in the world after Thailand. The changes of cropping practices into intensive systems leads rice crop to more pest and disease emergences. Blast, sheath blight, red stripe, and discolored grains are major problems for rice production. Discolored grains are observed in both dry and wet season, but more severe in wet season.

Symptoms of grain discoloration appears externally on the glumes or internally on the kernels, or both, the dots varied in size, shape and colors, and caused by a large number of fungi and bacteria associated (Ou 1983, Nghiem 1993). Discolored grains were

discovered in central provinces of Vietnam from 1991 (PPD-MPPC, 1996). In 1992, epidemics were found in Red River delta, midland and central coastal regions. The yield loss was approximately estimated from 20 to 50 per cent because of chaffy and unfilled-grains (Tuat 1997). In Mekong delta, surveys have been conducted at Cai Lay district, Tien Giang province, nine fungal species have been found, i.e. *Heminthosporium oryzae*, then *Fusarium moniliforme* and *Trichoconis padwickii* etc. (Hoang 1993).

Control of seed-borne fungi will increase percentage of seed germination and grain yield (DGISP 1985). Use of Dithan-45M and Ceresan restricted some seed-borne fungi (Dharam 1971, Mathur 1972). Anvil 5 SC with 1-1.5 litre per hecta applied at 5 to 10 days after flowering was recommended to control

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grain discoloration (Hai 1996), then Bonanza 100FL with 400cc per hecta reduced severity of the discolored glumes (Cuong 1998). Bacteria antagonists to treat the discolored grains obtained better effect on seed germination (Thach 1998).

Seed health project has been developed, series of experiments and surveys have been conducted to understand pathogens, yield loss and how to improve farmers' rice seeds in the delta.

MATERIALS AND METHODS

Seed samples were first collected from two different locations of Mekong delta as Long An and Can Tho provinces. In Long An, two main rice ecosystems of rainfed and irrigated culture types were selected. In Can Tho, seeds were collected in irrigated rice ecosystem. For sampling, we selected one kg of seeds / variety. In Long An, 60 seed samples of 12 varieties (local plus improved), while in Can Tho, only two most popular varieties IR50404 and IR64 were selected from 10 different rice farmers at four villages in Omon district.

Laboratory experiments: Survey of seed borne fungi and its effects on grain quality was carried out.

Observation of percent seed borne fungi of seed samples was conducted using Blotter method (Mathur and Olga Kongsdal 1994, Neergard 1988, Mew and Misra 1994). The simultaneous survey was also done by Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark. Samples of discolored grains with more than 25 % severity were submitted to seed quality laboratory of CLRRRI for gelatinization test (Little 1958), amylose content (Sadasivam and Manikam 1982), gel consistency (Cagambang 1973) and protein content (Microjeldal).

Field experiments: Effect of discolored grains with different severity levels to grain yield and yield components was conducted with IR64 seed samples.

Discolored grains of variety IR64 were carefully selected and classified into 0, 1, 3, 5, 7, 9 corresponding to healthy, 1, 1-5, 6-25, 26-50, 51-100 % of its infection, respectively (S.E.S 1996). These samples were transplanted under field condition at CLRRRI with healthy seed sample as control.

Experiment was laid out in a RCBD with six treatments and three replications. The following treatments were: (1) severity of discolored grains (scale 9; 51 - 100%), (2) severity of discolored grains (scale 7; 26 - 50%), (3) severity of discolored grains (scale 5; 6 - 25%), (4) severity of discolored grains (scale 3; 1 - 5%), (5) severity of discolored grains (scale 1; 1%), (6) severity of discolored grains (scale 0; 0%). Survey of seed-borne fungi were also conducted before seeding.

Data recording

1) Occurrence of seed borne fungi on 60 seed samples of 14 varieties in Long An province and 11 seed samples of two varieties in Can Tho province.

2) Effect of discolored grains to milling recovery (brown rice, white rice, head rice, chalkiness length, length/width ratio) and cooking quality (gelatinization, amylose content, gel consistency, protein content).

3) Percentage of seed germination, seedling height (cm), discolored grains, yield and yield components (1000 grain weight).

Statistical analysis

All variables were subjected to Analysis of Variance (ANOVA) using SAS (V. 6.11).

RESULTS

1) Survey of seed borne fungi

The presence of fungi was observed from 60 samples of 12 varieties including *Alternaria padwickii*, *Bipolaris oryzae*, *Cephalosporium oryzae*, *Curvularia oryzae*, *Fusarium gramineum*, *Phoma sorghina*, *Sarocladium oryzae*, *Tilletia barclayana*, *Ustilagoideia virens* in Long An. The highest percentage of infected seeds by *Alternaria padwickii* was 2.85-24.10%. The observation showed that seed infection percentage of improved cultivars was higher than landraces (Table 1). About 10-98 % of samples carried infection with these fungi. The highest percentage of infected seeds by *Alternaria padwickii* was noticed in Table 2.

Otherwise, there were eight fungal species from the seed samples of IR50404 in Can Tho as *Alternaria padwickii*, *Bipolaris oryzae*, *Fusarium moniliforme*, *Fusarium pallidoroseum*, *Fusarium subglutinans*, *Microdochium oryzae*, *Phoma sp.*, *Sarocladium oryzae* (Table 3). Only two fungal species of *Alternaria padwickii* and *Bipolaris oryzae* were found in IR64 (Table 4).

The highest percentage of infected seeds by recognized among these both IR64 and *Alternaria padwickii*, *Bipolaris oryzae* was IR50404.

Table 1: Infected seed percentage of common cultivars (local and improved) grown in the Mekong Delta by blotter method (1997 late wet season, Long An province)

Designation	Infected seeds (%)				
	A. p.	B. o.	C. spp.	F. g.	S. o.
1 IR64	22.60	7.05	18.30	2.55	4.30
2 IR9729	18.25	7.90	18.15	2.20	3.80
3 MTL110	24.10	9.95	19.05	0.95	2.30
4 IR52280	20.50	6.50	16.50	3.00	2.60
5 OM1706	17.95	5.15	17.25	2.00	2.20
6 IR1626	13.05	5.00	16.60	0.30	3.10
7 Nep sap	5.10	2.65	8.50	0.50	0.00
8 Nho do	2.85	2.80	5.80	0.25	0.00
9 Nangthom	4.35	3.25	4.35	0.85	0.15
10 Jasmin	10.10	5.80	5.50	0.65	0.80
11 Tainguyen	8.55	5.00	8.50	1.95	1.85
12 IR59066	17.60	5.60	18.50	2.90	2.60

(A. p.) *Alternaria padwickii*, (B. o.) *Bipolaris oryzae*, (C. spp.) *Curvularia spp.*, (F. g.) *Fusarium gramineum*, (S. o) *Sarocladium oryzae*

Table 2: Occurrence of seed borne fungi in rice seed samples and infected seeds by blotter method (1997 late wet season, Long An province).

Fungus	Infected samples (%)	Infected seeds (%)
<i>Alternaria padwickii</i>	98.00	12.00
<i>Bipolaris oryzae</i>	93.00	4.90
<i>Cephalosporium oryzae</i>	38.30	0.34
<i>Curvularia spp.</i>	95.00	13.44
<i>Fusarium gramineum</i>	60.00	1.50
<i>Phoma sorghina</i>	18.00	0.10
<i>Sarocladium oryzae</i>	48.30	1.90
<i>Tilletia barclayana</i>	10.00	0.16
<i>Ustilagoidea vires</i>	10.00	0.05

Data collected from 60 samples

Table 3: Occurrence of seed borne pathogens in seed samples of IR50404 (1998 dry season, Can Tho province)

Seed sample	Seed borne fungi in seed samples (%)							
	A.p	B.o	F.m	F.p	F.s	M.o	P.sp	S.o
1 (IR50404), F. A*	0.50	1.00	-	-	-	-	-	-
2 (IR50404), F. B	0.50	0.50	-	-	0.50	-	-	-
3 (IR50404), F. C	28.00	3.00	-	-	-	-	-	-
4 (IR50404), F. D	25.00	0.50	-	-	-	-	-	-
5 (IR50404), F. E	21.00	3.50	-	1.00	-	-	-	-
6 (IR50404), F. F	6.50	1.00	4.50	1.50	-	-	-	-
7 (IR50404), F. G	12.50	11.50	1.50	4.50	-	0.50	-	0.50
8 (IR50404), F. H	4.50	2.50	1.00	-	-	-	-	-
9 (IR50404), F. I	8.50	1.00	-	-	-	-	-	-
10 (IR50404), F. J	8.00	36.00	-	-	-	-	0.50	-

* F. A: Farmer A to Farmer J. (A.p) *Alternaria padwickii*, (B.o) *Bipolaris oryzae*, (F.m) *Fusarium moniliforme*, (F.p) *Fusarium pallidoroseum*, (F.s) *Fusarium subglutinans*, (P.sp) *Phoma sp.*, (M.o) *Microdochium oryzae*, (S.o) *Sarocladium oryzae*.

Table 4 : Seed borne fungi percentage of variety IR64 (1999 Dry season, Can Tho province)

Seed sample	Seed borne fungi in seed samples (%)							
	A.p	B.o	F.m	F.p	F.s	M.o	P.sp	S.o
1 IR 64 (51–100%)	4.0	0.5	-	-	-	-	-	-
2 IR 64 (26 – 50%)	4.0	0.0	-	-	-	-	-	-
3 IR 64 (6 – 25%)	3.0	2.0	-	-	-	-	-	-
4 IR 64 (1 – 5%)	3.5	0.0	-	-	-	-	-	-
5 IR 64 (1 %)	4.5	1.0	-	-	-	-	-	-
6 IR 64 (Healthy)	2.0	0.0	-	-	-	-	-	-

(A.p): *Alternaria padwickii*, (B.o): *Bipolaris oryzae*

2) Effects of grain discoloration to rice grain quality

An observation on effects of grain discolored (in terms of more than 25 % severity) to rice milling recovery indicated that all parameters of brown rice, white rice, head rice, brown rice length and length-width ratio were significantly affected as compared to healthy samples (Table 5). About cooking and eating quality, amylose content, gel consistency (GC) and protein content (PrC) were also found to be affected as compared to healthy samples. Amylose content in IR64, Nang thom and IR9729 increased higher in discolored seeds. Gel consistency in

discolored seeds of IR64 and Nang Thom was lower value as compared to healthy seeds while other varieties, discolored seeds had higher GC than healthy seeds. Protein content value of discolored seeds in all varieties was significantly higher than healthy seeds (Table 6). This is just a preliminary survey on quality of severe discolored grains, more researchs are needed in order to confirm which factors of grain quality are most affected when infected seeds caused by a complex of seed borne fungi. Effect of grain discoloration to 1000-grain weight differed 3-5 grams in all cultivars (Table 7).

Table 5: Effects of grain discoloration to rice milling recovery.

Seed sample	Brown rice (%)	White rice (%)	Head rice (%)	Chalkiness (%)	Length (mm)	Length/Width
1 Jasmine (H)	77.90 c	70.95 b	58.17 a	5.00 c	7.05 b	3.36 cd
2 Jasmine (I)	75.91 d	66.35 d	52.61 b	5.00 c	6.83 c	3.25 e
3 IR59606 (H)	79.02 bc	71.62 ab	58.50 a	10.00 c	6.77 cd	3.22 e
4 IR59606 (I)	76.44 d	65.73 d	50.96 b	10.00 c	6.64 d	3.26 e
5 IR64 (H)	79.66 ab	72.03 ab	45.52 c	5.00 c	7.25 a	3.54 a
6 IR64 (I)	61.03 e	53.02 e	39.48 d	15.00 b	7.16 ab	3.43 dc
7 Nangthom(H)	79.12 bc	68.73 c	39.65 d	60.00 a	6.26 e	3.22 e
8 Nangthom (I)	77.77 c	65.76 d	40.70 d	60.00 a	6.12 e	3.22 e
9 IR97219 (H)	80.85 a	73.30 a	50.82 a	15.00 b	7.26 a	3.56 a
10 IR9729 (I)	78.28 bc	66.74 d	46.26 c	15.00 b	7.12 ab	3.48 ab
CV (%)	0.91	1.36	12.27	1.20	1.41	-

Values in the same column followed by the same letter are not significantly different (Duncan's test $P < 0.05$) (H): healthy, (I): infected

3) Effect of discolored grains to growth and yield

Pathogens borned in discolored seeds of IR64 were identified as *Alternaria padwickii* and *Bipolaris oryzae*. These seeds were used as source for field experiment. Results from this experiment shows in Table 8, there were significantly different of seed germination (%), seedling height (cm). This also indicates that at first stage of rice crop, these two

parameters were most affected. However, at harvest time, dirty grain percentage and yields were not significantly different among treatments. Dirty grains of succeeding crops might be also affected by other factors such as relative humidity (%) during flowering stage. Grain yield was not differently obtained among treatments.

Table 6: Effect of grain discoloration to cooking and eating quality

Seed sample	Alkalinization (scale)	Amylose content (%)	Gel consistency (mm)	Protein content (%)
1 Jasmine (H)	7	20.98 d	51.00 cd	8.12 c
2 Jasmine (I)	7	21.53 cd	53.33 cd	9.20 a
3 IR59606(H)	4	21.53 cd	46.00 d	7.67 d
4 IR59606(I)	4	22.90 c	54.67 cd	8.90 b
5 IR64 (H)	5	26.46 ab	71.00 a	7.46 de
6 IR64 (I)	5	27.28 ab	59.67 bc	7.24 ef
7 Nangthom(H)	6	25.91 b	71.33 a	6.83 g
8 Nangthom(I)	5	28.10 a	66.50 ab	7.09 fg
9 IR97219 (H)	5	22.35 cd	48.67 d	8.12 c
10 IR9729 (I)	4	25.91 b	51.33 cd	8.71 b
CV (%)	--	3.72	88.1	2.13

Values in the same column followed by the same letter are not significantly different (Duncan's test P<0.05)
(H): healthy, (I): infected

Table 7. Effect of grain discoloration to 1000 grain weight as compared to healthy seeds

Seed sample	1000-grain weight (gr)		Infected seed (%)
	Discolored grain	Healthy grain	
Nep Sap	27.93	32.11	20.47
Tai Nguyen	18.40	19.30	10.23
IR 9729	23.16	27.29	18.10
Nang thom	18.98	21.30	11.43
IR 59606	21.73	25.23	24.07
IR 64	23.74	27.83	27.13
Jasmine	26.49	28.81	26.13
OM 1706	24.34	27.66	33.53
Average	22.64	25.90	21.39

Grain discolored with more than 25 % severity is selected (SES, 1996).

Table 8: Effect of grain discoloration to seed germination, seedling height, dirty grains and yield of IR 64 (1999 dry season, Omon, Can Tho, Vietnam)

Discolored seed samples	Seed germination (%)	Seedling height (cm)	Discolored grain (%)	Yield (T/ha)
1 IR 64 (51-100%)	17.28 d	7.67 c	20.67 a	6.00 a
2 IR 64 (26- 50%)	38.00 c	13.67 c	17.00 a	6.20 a
3 IR 64 (6- 25%)	38.96 c	16.67 bc	15.67 a	6.20 a
4 IR 64 (1- 5%)	54.57 b	29.67 abc	15.33 a	6.17 a
5 IR 64 (1%)	65.75 b	41.67 ab	13.33 a	6.47 a
6 IR 64 (control)	87.96 a	47.67 a	16.33 a	6.67 a
CV (%)	17.00	54.49	35.73	7.79

Values in the same column followed by the same letter are not significantly different (Duncan's test P<0.05)

CONCLUSIONS

Survey on discolored grains of 12 varieties collected from Long An province showed that there were nine fungal species with detection frequency of infected seeds: *Cuvelaria spp* (13.44%), *Alternaria padwickii* (12%), *Bipolaris oryzae* (4.9%), *Sarocladium oryzae* (1.9%), *Fusarium gramineum* (1.5%), *Tilletia barclayana* (0.16), *Phoma sorghina* (0.1%), *Cephalosporium oryzae* (0.34%), *Ustilaginoidea virens* (0.05%). In Can Tho province, eight fungal species were found in seed samples of IR50404 and IR64, the fungi *Alternaria padwickii*, *Bipolaris oryzae*, *Fusarium moniliforme*, *Fusarium pallidoroseum*, *Fusarium subglutinans*,

Microdochium oryzae, *Phoma sp.*, *Sarocladium oryzae* were identified. Similar results were obtained in two different provinces. Based on this survey, we can noted that discolored grains in Mekong delta caused by a complex of fungi in which *Alternaria padwickii* and *Bipolaris oryzae* were the most popular ones, the presence of other species possibly depended on given variety and season. Seed infection percentage in improved cultivars was often higher than in landraces. The study also indicated that grain discoloration affected to rice milling recovery (brown rice, white rice, head rice, chalkiness, length, length/width ratio), cooking and eating quality (gelatinization temperature, amylose

content, gel consistency, protein content). Seed germination percentage, seedling height were obviously affected by discolored grains. In field experiment, one component of yield i.e. 1000-grain weight was mostly affected. However, grain yield was not significantly different among the treatments. More surveys on seed borne diseases and yield loss due to discolored grains of direct seeding practice need to be conducted in order to suggest for

management procedures to produce high quality seed in the Mekong delta.

Acknowledgements

The authors thank to Madam Olga Kongsdal at the Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark, for her kind cooperation in this research work.

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SUMMARY IN VIETNAMESE**Nấm gây bệnh lem hạt, ảnh hưởng hạt lem đối với phẩm chất hạt trên một số giống lúa ở ĐBSCL**

Nghiên cứu bệnh lem lép hạt được tiến hành trên một số giống trồng đại trà từ năm 1997, kết quả ghi nhận như sau; kết quả điều tra về thành phần nấm bệnh trên hạt có 9 loài nấm hiện diện trên 60 mẫu hạt trên 12 giống lúa (mùa và cải thiện) thu thập tại tỉnh Long an, bằng phương pháp "blotter", *Curvularia* spp. có tần suất xuất hiện cao nhất (13,44%), kế đến là *Alternaria padwickii* (12%), *Bipolaris oryzae* (4,9%), *Sarocladium oryzae* (1,9%), *Fusarium gramineum* (1,5%), *Tilletia barclayana* (0,16), *Phoma sorghina* (0,1%), *Cephalosporium oryzae* (0,34%), *Ustilagoideae virens* (0,05%). Tại tỉnh Cần Thơ, 20 mẫu của 10 hộ nông dân, trên 2 giống IR50404 và IR64 có 8 loài nấm lưu tồn trên hạt, trong đó *Alternaria padwickii* có tần suất xuất hiện cao nhất, kế đến là *Bipolaris oryzae*, *Fusarium moniliforme*, *Fusarium pallidoroseum*, *Fusarium subglutinans*, *Microdochium oryzae*, *Phoma* sp., *Sarocladium oryzae*. Nhìn chung thành phần nấm tại 2 địa điểm điều tra không khác nhau nhiều. Kết quả điều tra còn cho thấy các giống lúa cải tiến có tỷ lệ nấm bệnh xuất hiện cao hơn các giống lúa địa phương. Về ảnh hưởng của bệnh lem hạt đối với phẩm chất hạt giống như, phẩm chất xay chà, phẩm chất cơm, tỉ lệ nảy mầm hạt lem, chiều cao cây mạ, năng suất và các yếu tố năng suất (TL 1000 hạt) cho thấy khi bị bệnh lem hạt thì các chỉ tiêu trên đều bị ảnh hưởng rất rõ. Tuy nhiên về năng suất thì không khác biệt có ý nghĩa đối với lúa cấy, khi sử dụng hạt lem làm mạ. Do vậy để đánh giá về thiệt hại năng suất và các biện pháp phòng trừ hạt, cần tiến hành thêm các thí nghiệm trên lúa sạ thẳng. Điều tra xác định thành phần nấm và vi khuẩn ký sinh trên hạt sẽ được tiếp tục với số lượng mẫu lớn hơn cũng như tiến hành các biện pháp xử lý hạt nhằm giúp bà con nông dân trong khâu quản lý hạt giống được tốt hơn.
