~~RICE~~ GRAIN DISCOLORATION IN COLOMBIA

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"The realization that deterioration occurs in all forms of life and leads ultimately to death is not at all comforting".

Baki and Anderson

Since rice is not propagated vegetatively the perpetuation of the crop depends on the sexual seed production. Today that our agriculture has reached a high level of technification it is imperative to obtain good quality of seed. Some of the common attributes considered to guarantee the obtention of good quality of seed are: germination, vigor, physical and genetic purity, humidity, mixture of seeds from other crops and mechanical injury. However, seeds are both victims and efficient vehicles of diseases. Commercial seed lots have highly variable percentages of infected seeds ranging from a fraction of a percent to 100%, but well-cleaned seed usually has less than 5% seed pathogens transport (Baker, 1979).

Rice grains may be infected by various microoganisms before or after harvest, causing grain discoloration. Damage caused by seed-borne pathogens varies from small brown spots to complete browing of the glumes. The problem frequently extends to the endosperm and even to the embryo (Figure 1).

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It is common to observe a high percentage of unfilled grain and low germination as a consequence of a complete destruction of the floral organs. The presence of spotted or stained Kernels reduces the grade of rice. Grains that are spotted severily and therefore chalky, break into pieces in the milling process; thus, Kernel spot reduces yield of head rice (Atkins and Marchetti, 1979). Spotted or infected seeds may provide a means for pathogens to survive from one generation of host plants to the next, and dissemination from one geographical area to another.

In recent years grain discoloration has received increased attention in tropical rice production areas. The principal causal agent of this disease differs from area to area. In Nigeria the most common fungus isolated from discolored seed is Sarocladium attenuatum, syn. Acrocylindrium oryzae (IITA, 1982); in the Philippines it is Trichoconis sp., syn Alternaria sp. (IRRI, 1976, 1977) and in Colombia the most common pathogen is Cochliobolus miyabeanus, conidial stage Helminthosporium (Bipolaris) oryzae. Here and elsewhere the disease is associated with different complexes of rainy weather, high relative humidity, upland conditions, infertile soils and the hoja blanca virus. Therefore rice grain discoloration is a complex problem determined by the interaction of host pathogen and environment. Modern man has bred plants for increased yield, easier harvesting, minimal dormancy, softer seed coat, and better nutrition, but is leaving the rice crop vulnerable to seed deteriotion prior to harvest as well as during storage.

FUNGI ASSOCIATED WITH GRAIN DISCOLORATION

Seeds of six varieties and lines of rice harvested in an area with high incidence of grain discoloration were used. Four hundred seeds per variety or line were incubated following the Agar plate test and the 2,4-D blotter test (Figure 2). Fourteen different genera of fungi and some unidentified bacteria were isolated (Table 1) CICA 4 and line 16130, both showing resistance to grain discoloration in the field, had the lowest incidence of fungus growth in comparison to the other four varieties and lines which showed an incidence of fungus growth higher than 90 per cent. only three species, Cochliobolus miyabeanus, conidial stage Helminthosporium (Bipolaris) oryzae; Metasphaeria albescens, conidial stage Rhynchosporium oryzae; and Phyllosticta sp., were the most common fungi isolated from discolored grains. However, Helminthosporium (Bipolaris) oryzae was consistently the most frequent fungus isolated even from those varieties showing resistance to grain discoloration in the field.

PATHOGENICITY TESTS

Seven fungi isolated from the endosperm of discolored grains, and four varieties showing different degrees of resistance to grain discoloration in the field were used. Three methods of inoculation were employed: a) injecting the conidial suspension at the heading stage b) spraying at panicle emergence and c) spraying after flowering (Table 2). The tests were carried out in plastic chambers (3.0 x 3.0 x 2.2 m. high) located in a greenhouse equipped with mist sprayers to maintain high relative humidity. Three weeks after inoculation a detailed observation of developed symptoms was

Table 1. Microorganisms isolated from the endosperm of moldy grains (%).

Microorganisms	Variety or line [a]					
	16130 [b]	16240 [c]	16259 [c]	16260 [c]	CICA 4 [b]	CICA 8 [c]
Helminthosporium oryzae	17	83	72	75	28	77
Rhynchosporium oryzae	4	8	1	2	1	2
Phyllosticta sp	2	16	3	5	1	1
Alternaria padwickii	1	—	3	1	1	—
Curvularia spp.	—	—	1	1	3	1
Helicoceras oryzae	2	—	2	—	2	—
Fusarium spp	—	2	—	—	—	—
Pyricularia oryzae	—	—	—	—	—	1
Chaetomium sp.	—	—	2	—	—	—
Nigrospora oryzae	—	—	—	2	—	—
Oidium spp.	—	1	—	—	—	—
Cladosporium spp	1	—	—	—	—	—
Penicillium spp.	3	—	—	—	1	—
Aspergillus spp.	2	—	—	—	—	—
Bacterial	5	—	8	4	8	2
Unidentified non-sporulating	28	2	11	9	20	12
With fungus growth	67	99	96	92	54	92
No fungus growth	33	1	4	8	46	8

a. Seed harvested in an area with high incidence of grain discoloration.

b. Variety or line showing resistance to grain discoloration in the field.

c. Variety or line showing susceptibility to grain discoloration in the field.

Table 2. Pathogenicity tests of seven seed-borne fungi on four different rice varieties.

Method of inoculación	Variety or line	DG rating [a]	H. oryzae	Rh. oryzae	Curvularia sp.	A. Padwickii	Fusarium sp.	Phyllosticta sp.	Chaetomium sp.	Control
Injection at heading stage[c]	CICA 4		5	5	5	1	7	1	3	1
Spray at panicle emergence[d]	CICA 4	1	9	5	7	3	5	5	7	1
Spray after flowering[e]	CICA 4		5	1	5	1	1	3	3	1
Injection at heading stage	CICA 8		7	1	5	3	3	3	3	1
Spray at panicle emergence	CICA 8	9	9	9	5	9	9	9	9	1
Spray after flowering	CICA 8		5	1	5	1	9	3	5	1
Injection at heading stage	Metica 1		5	7	7	3	5	5	1	1
Spray at panicle emergence	Metica 1	7	9	9	7	7	9	5	7	1
Spray after flowering	Metica 1		5	3	5	1	5	5	3	1
Injection at heading stage	16130		5	5	7	3	5	3	5	1
Spray at panicle emergence	16130	1	9	9	7	7	9	9	9	1
Spray after flowering	16130		5	3	5	1	7	5	7	1

a. DG = discolored grain; 1 to 9 scale, where 1 = absence of discoloration; 9 = severe discoloration.

b. H. = Helminthosporium; Rh. = Rhynchosporium; A. = Alternaria.

c. 90 days after planting.

d. 100 days after planting.

e. 110 days after planting.

recorded. All the different isolates induced grain discoloration; the severity of the symptoms depended on the isolate and the method of inoculation (Table 2). Helminthosporium (Bipolaris) oryzae and Curvularia sp. consistently induced the most severe symptoms, as did spraying at panicle emergence. This confirms the earlier results obtained by Castaño (1972, unpublished data). He doing pathogenicity tests with both H. oryzae an Curvularia sp. got similar symptoms to those obtained above. Both fungi induced brown necrotic lesions on the lemma and palea, and also a high per cent of grain sterility. In addition, H. oryzae induced strong brown necrotic lesions on the flag leaf sheath. On the other hand Curvularia sp. only affected the glumes of the grains.

EFFECT OF GRAIN DISCOLORATION ON GERMINATION

One thousand one hundred seeds of each five lines harvested in two different locations were incubated following the Agar plate test. Lines 16240, 16258, 16259 and 16260 are susceptible to grain discoloration while line 11963 is resistant (Table 3). Decreases in germination in the susceptible lines ranged from 26 to 41 per cent while the resistant line had 1 per cent reduction in germination. The Duncan's Multiple range test shows that when the seed is harvested in an area free of grain discoloration its germination is very high (Table 4). Since the line 11963 is resistant to grain discoloration in the field, its germination was not affected in the area having heavy disease incidence. Severity of grain discoloration was directly related to increased fungus growth and to lower germination (Table 5). In susceptible varieties the fungus growth is usually higher than 90 per cent which reduces the germination significantly.

Table 3. Effect of grain discoloration on germination.

		Percentage of germination in:	
Line	Discolored grain ratings [a]	Discolored seed [b]	Clean seed [c]
16240	9	51	86
16258	9	45	86
16259	9	54	80
16260	9	43	82
11963 [d]	1	96	97

a. 1 to 9 scale, where 1 = absence of discoloration; 9 = severe discoloration.

b. Discolored seed harvested in an area with high disease incidence.

c. Clean seed harvested in an area free of grain discoloration

d. Line showing resistance to grain discoloration in the field.

Table 4. Duncan's multiple range test for germination of five lines of rice harvested at two different locations.

Line	Origen	Discolored grain ratings [a]	Mean [b]
11963[c]	CIAT[d]	1	97 A
11963	La Libertad [e]	1	96 A
16263	CIAT	1	86 AB
16240	CIAT	1	86 B
16259	CIAT	1	82 B
16260	CIAT	1	80 B
16259	La Libertad	9	54 C
16240	La Libertad	9	51 C
16258	La Libertad	9	45 C
16260	La Libertad	9	43 C

a. 1 to 9 scale, where 1 = absence of discoloration; 9 = severe discoloration.

b. Mean of 1100 seeds.

c. Line showing resistance to grain discoloration in the field.

d. Area free of grain discoloration.

e. Area with severe grain discoloration.

Table 5. Effect of fungus growth on germination of rice seeds.

Line	Discolored grain ratings [a]	Fungus growth (%)	Germination (%)
16240	9	94 [b]	51
16258	9	99	45
16259	9	98	54
16260	9	98	43
11963	1	62	96

a. 1 to 9 scale, where 1 = absence of discoloration; 9 = severe discoloration.

b. Mean of 1100 rice seeds obtained from an area with high incidence of grain discoloration; r = - 0.94.

A high correlation between fungus growth and germination exists ($r = -0.94$) In resistant 11963 the fungus growth was relatively high (62 per cent) but germination was not affected. Most of the fungi observed on seeds of resistant varieties are usually weak pathogens which cause little grain discoloration and, therefore, do not reduce germination.

EFFECT OF GRAIN DISCOLORATION ON SEEDLING DEVELOPMENT

Four hundred seeds of the variety CICA 8 were harvested in an area of high incidence of grain discoloration. The seeds were separated in five classes according to the severity of the disease. The seed was planted in sterilized soil under greenhouse conditions. Seven days after planting it was measured seedling size. The reduction in seedling size ranged from 17 per cent to 40 per cent, and the contribution of classes 7 and 9 in decreasing seedling size was higher than 30 per cent (Table 6). It is clear that grain discoloration disease affects seedling development significantly and decrease in seedling size is in close association with increasing severity of grain discoloration (Figura 3).

EFFECT OF GRAIN DISCOLORATION ON SOME YIELD COMPONENTS

Ten panicles representing each grade severity of grain discoloration were selected from an area of high incidence of the disease. The grain yield of the rice plant is known to be a function of the number of panicles per plant, the number of filled grains per panicle and the mean weight of individual grains. Grain development was adversely affected by heavy infection (Table 7). From this table it is clear that the main contributing

Table 6. Duncan's multiple range test for seedling size as affected by grain discoloration disease.

Discolored grain ratings [a]	Number of seedlings per class	Mean (cm)	Seedling size reduction (%)
1	92	4.8 A	-
3	94	4.0 B	17
5	66	3.9 B	19
7	92	3.3 C	31
9	58	2.9 C	40

a. 1 to 9 scale, where 1 = absence of discoloration; 9 = severe discoloration.

factor to yield loss was the development of fewer grains per panicle. Compared with grade 1, significante reduction in grain number of 38 per cent and 40 per cent was caused by severities 7 and 9 respectively. The other factor was the incidence of empty glumes and incompletely filled grains. Although only 7 per cent of the total grains remained empty on grade 1, up to 34 per cent were empty on grade 9. On the whole, the contribution of empty glumes to yield loss was 15 per cent on grade 3; 16 per cent on grade 5; 29 per cent on grade 7 and 34 per cent on grade 9 (Figure 4). Severe grain discoloration (grades 7 and 9) were also found to produce a high proportion of "partially" filled grains as reflected in reduced 100-grain weight which significantly contributed to yield loss by 14 per cent and 16 per cent on grades 7 and 9, respectively (Figure 5). In general, there is a high correlation between the frequency of unfilled grains and weight loss. That is, there is a high correlation between the incidence of grain discoloration and seriousness of the attack. Unfilled grains are usually severely stained.

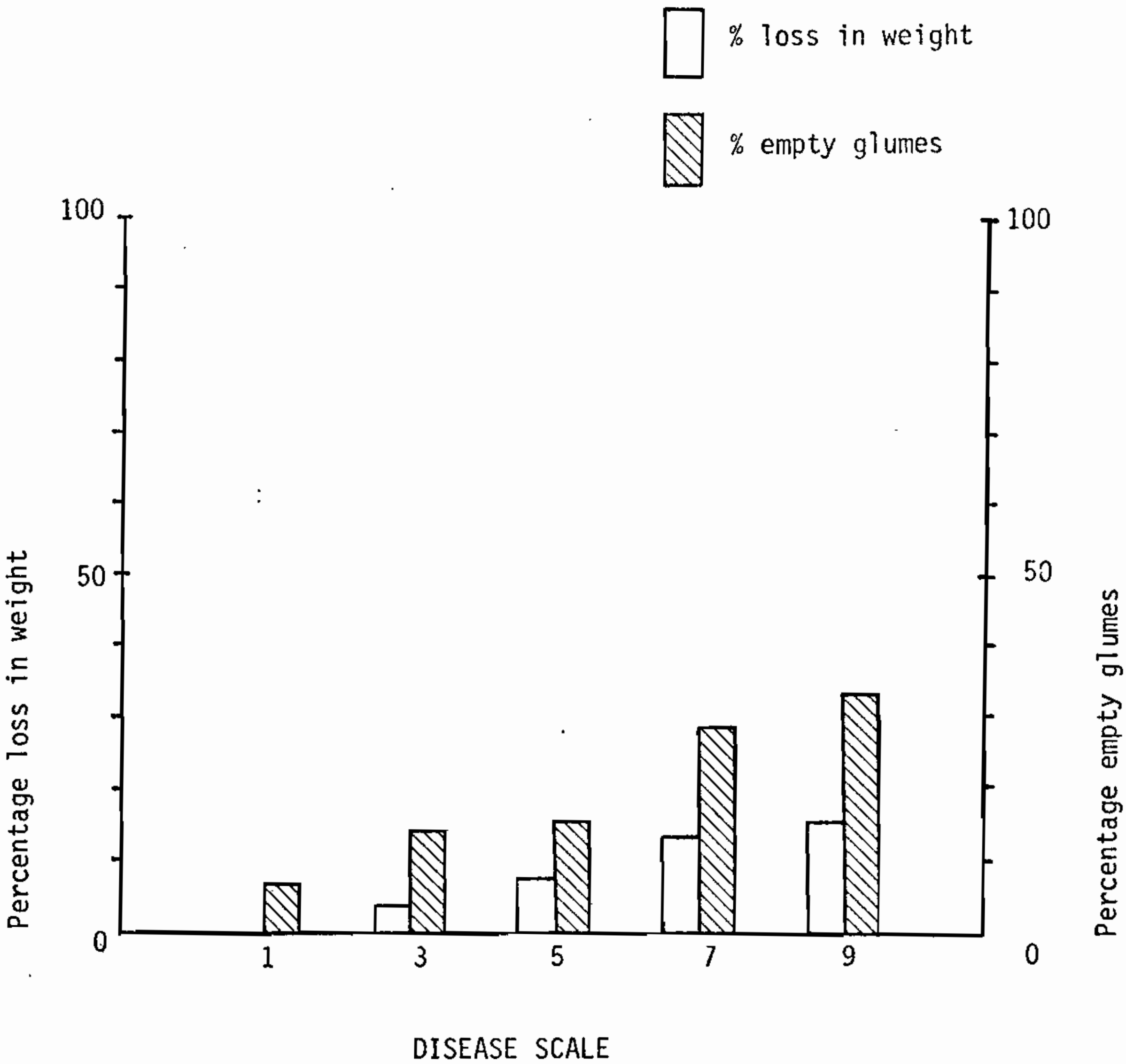

Figure 4. Loss in grain weight as affected by grain discoloration disease.

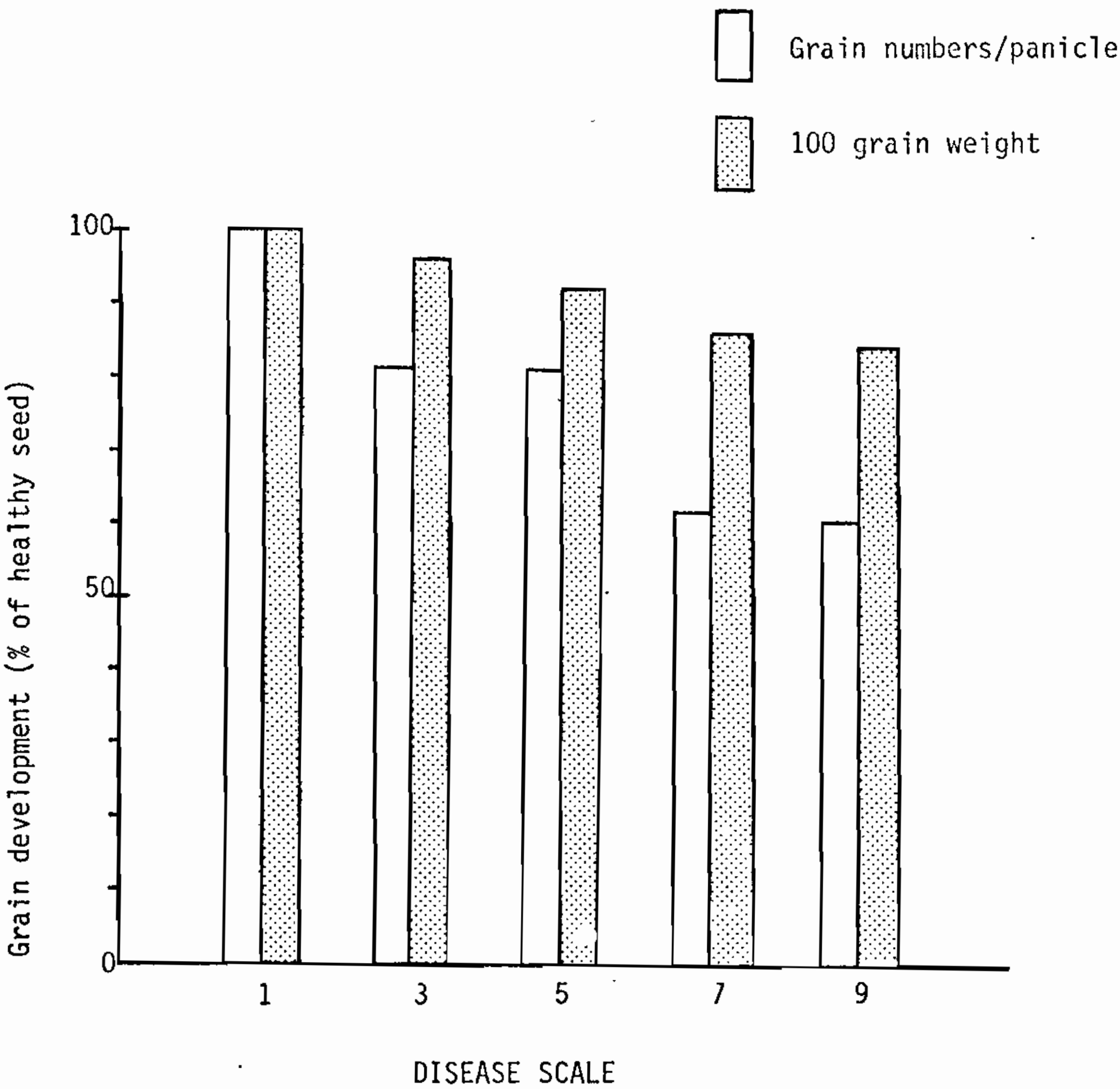

Figure 5. Grain development as affected by grain discoloration disease

Table 10. Association between grain discoloration and hoja blanca virus in 75 varieties.

Discoloration grain Scale [a]	Upland rice			Lowland rice		
	Varieties with grain discoloration [b] (No.)	Hoja blanca		Varieties with grain discoloration (No.)	Hoja blanca	
		Varieties (No.)	Incidence (%)		Varieties (No.)	Incidence (%)
1	0	0	0	6	4	67
3	36	23	64	45	30	67
5	27	22	81	14	13	93
7	12	11	92	10	10	100

a. 1 to 9 scale, where 1 = Clean; 9 = severe grain discoloration.

Table 12. Escala para evaluar germoplasma de arroz afectado por el manchado de grano.

CLASE		INDICE
Laboratorio	Campo	
1[a]	1	1
2		5
3	3	10
4		15
5	5	30
6		45
7	7	60
8		80
9	9	100

a. Escala de 1 a 9 en donde 1 = grano limpio; 9 = grano severamente manchado.

$$ASA\ (\%) = \frac{\varepsilon\ (n \times I)}{N}$$ en donde,

ASA (%) = Area de semilla manchada en porcentage.

n = Número de panículas clasificadas en cada clase

I = Indice de severidad por cada clase

N = Número total de panículas observadas