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Evaluation of seed health of some rice varieties under different conditions

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Abstract. An experiment was conducted at the International Rice Research Institute (IRRI) to evaluate the seed quality of some rice varieties under different treatment conditions. Significant variation in respect of germination (%) and pathogenic infection was observed in different varieties and treatments. Among the varieties, C-4/Malaqkit (V1) and IR59 (V2) had the higher % of germination in all treatments except chemical treatment (T2). In case of pathogen, *Alternaria padwickii* was dominant, and its incidence was the highest in best seed (T1) & Farmer's original seed (T3) for all varieties except R30 (V4). There was no pathogen in chemically treated seeds. For both in-soil and in-between paper germination test, V1 had the highest and V4 had the lowest percent of normal seedlings in all the treatments. The percent of abnormal seedlings of all varieties was statistically similar in in-soil test but dissimilar for in-between paper test. The percent of abnormal seedlings in between paper test and dead seeds for in-soil test were higher in case of V2 & V4. Variety V2 had the highest while V4 had the lowest vigor in all treatments. Among the treatments, the vigor and normal seedling were the highest in T1 irrespective of varieties. The highest lethal seed infection was caused by *Fusarium moniliforme. Alternaria padwickii* and *Curvularia* spp. also caused lethal seed infection.

Keywords: Lethal seed infection; Rate of germination index; Seed health; Seed vigor.

Introduction

Seed health refers primarily to the presence or absence of disease-causing organisms such as fungi, nematodes, bacteria, viruses and insects associated with seed. Physiological conditions such as trace element deficiency may also affect seed health (ISTA, 1985). Farmers often use seeds that have impurities and contaminants and are infected with pathogens (Fujisaka et al., 1993)

The importance of seed quality in realizing the full potential of a variety is well known. The three major aspects of seed quality are a) genetic and physical purity, b) high germination percentage and vigor, and c) free from seed-borne diseases and insects (Seshu and Dadlani, 1989). Seed vigor is recognized as an important seed quality parameter distinct from germinability (Seshu et al., 1988).

The present investigation was carried out to establish whether seed-borne fungi are responsible for seedling abnormalities, the possibility of combining germination tests and seed health tests to determine the effect of seed treatments and seed processing procedures on germination and seedling vigor.

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Materials and Methods

The experiment was conducted at the International Rice Research Institute (IRRI), Philippines in the greenhouse and at the Rice Seed Health laboratory, where RCB design was followed with 3 replications. Four varieties (viz. V1 = C-4/Malaqkit, V2 = IR59, V3 = IR 54 and V4 = R30) were selected for the experiments. Seed from each variety was grouped into three categories: i) Manually sorted best quality seed from Farmer's original seed (T1), ii) Original farmers' seeds treated with Benlate @ 0.3% and Dithane-M-45 @ 0.3% (T2) and iii) Farmers' original seeds (T3). Based on such categories, all four varieties were tested for associated microflora, germination, vigor, and lethal seed infection with the following tests.

Blotter Test

Two layers of blotting papers moistened with distilled water were placed inside the plastic plates to facilitate the penetration of near ultra-violet (NUV) light. From the working sample (400 seeds), 25 seeds per plate were placed and incubated at 22°C under alternate 12 h NUV light and 12 h dark cycle for 7 days. The seeds were examined under a microscope to evaluate fungal pathogens. For detection of associated nematodes, seeds were placed on a 10 cm diameter, 40 × 40 mesh steel wire dish filled

in the Baermann's funnels with about 250 ml water separately. The setup was incubated for 48 h. Approximately 20 ml of the water was collected into a small petri desh through loosening the rubber tube attached to the funnel, and nematodes were counted under a stereo binocular microscope.

Germination Test

In between paper method. Two sheets of square blotters (AGF 725-230 × 265 mm) were wetted by 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. Five and 9 days after sowing (DAS), all the normal seedlings were counted and removed from the paper. On 14 DAS, normal, abnormal, diseased seedling, and dead seeds were counted and placed separately in plastic petri dishes to examine the lethal seed infection.

In-soil method. From each sample 400 seeds were sown in line in the soil (1-2 cm depth) @ 100 seeds/tray and were kept in the glasshouse. Shoots rising more than 2 mm above soil surface were counted as germinated. Data were taken at 5 DAS and 9 DAS only for germination and on 14 DAS (as final reading) normal, abnormal, diseased seedlings and dead seeds were counted.

Seedling Vigor Test

For this, 100 seeds of each variety and treatments were taken in 3 replications in each petri dish and 10 ml of distilled water was added with seeds. Then the petri dishes were kept in the germination room. Data were taken at 3 DAS and 7 DAS to find out the rate of germination index (RGI) using the following formula:

RGI = (No. of seeds germinated at 3DAS / No of seeds germinated at 7 DAS) × 100

The seed samples were categorized by using the following scale of RGI value:

Higher vigor seeds: 81-100%, medium vigor: 71-80%, and low vigor: less than 70 (Malabanan, 1993).

Lethal Seed Infection

In the case of the in-between paper test, the abnormal (physiological, diseased and decayed and dead) seeds were counted and placed in petri dishes separately for the blotter test. After 4 days of incubation, seeds were examined under a sterio-microscope to confirm whether the abnormality or death of the seed was due to pathogens. If an adequate amount of pathogenic organisms was present near the embryo or in the middle portion of the seed or if a huge amount of necrotropic organism was present all over the seed, a lethal seed infection was considered to have occurred. The number of seeds or seedlings that died due to lethal seed infection was also noted.

Results and Discussion

Blotter Test

Significant variations with respect to percent germination and pathogenic infection were observed in different varieties and treatments (Table 1). Among the varieties, V1 and V2 had a significantly higher percentage of germination in T1, and V1 had the higher percentage of germination in all treatment levels. Percent germination was lower for chemically treated seed (T2), which may be due to chemical effects on the seed. In the case of pathogen, the percent of *Alternaria padwickii* was the highest in T1 (ranging from 1.33 to 44.0%) and T3 (ranging from 1.0 to 36.6%) for all varieties except V3. Other pathogens like *Curvularia* sp, *Fusarium moniliforme*, *Sarocladium oryzae*, *Pyricularia oryzae*, *Bipolaris oryzae*, *Microdochium oryzae* were also present on the seeds in T1 & T3, but there were no such patho-

Table 1. Germination and detection level of seed-borne fungi in 4 varieties and 3 seed categories.

Treat	Ger	CS	Ap	Cur	Fm	So	Po	Bo	Mo
V1T1	99.33 a	13.00 cd	44.00 a	4.33 bc	0.33 c	1.33 d	0.00 a	0.66 b	1.33 a
V1T2	95.66 ab	98.33 a	0.00 d	0.33 d	0.00 c	0.00 d	0.00 b	0.00 b	0.00 b
V1T3	93.67 ab	22.00 cb	36.66 b	36.66 b	1.00 c	4.66 d	0.33 b	0.33 b	1.33a
V2T1	99.33 a	35.66 bc	18.00 b	2.33 cd	1.33 c	1.33 c	1.66a	1.66 a	0.66 ab
V2T2	94.33 ab	99. 66 a	0.00 d	0.00 d	0.00 c	0.00 d	0.00 b	0.00 b	0.00 b
V2T3	82.00 c	29.33 c	8.33 c	5.00 b	4.00 b	2.66 b	1.00 ab	0.66 b	0.66 ab
V3T1	69.00 d	11.00 d	40.33 ab	3.66 c	1.00 c	4.00 a	0.00 b	0.33 b	0.66 ab
V3T2	47.33 e	16.66 cd	0.00 d	0.00 d	0.00 c	0.00 d	0.00 b	0.00 b	0.00 b
V3T3	59.00 d	8.00 d	43.66 ab	6.33 bc	2.33 bc	7.00 b	1.66 a	0.66 b	1.00 a
V4T1	91.66 b	38.33 bc	1.33 d	6.63 bc	2.33 bc	0.66 cd	0.00 b	0.53 b	0.33 b
V4T2	72.33 d	98.00 a	0.00 d	0.00 d	0.00 c	0.00 d	0.00 b	0.00 b	0.00 b
V4T3	64.33 d	49.33 b	1.00 d	21.33 a	9.00 a	0.66 cd	0.33 b	0.66 b	0.66 ab
5%lsd	7.65	17.65	5.73	2.41	2.67	1.01	1.21	0.96	0.72

In a column, means followed by a common letter are not significantly different at the LSD 5%. Ger: Germination; CS: Clean seed; Ap: *Alternaria padwickii*; Cur: *Curvularia* spp.; Fm: *Fusarium moniliforme*; So: *Sarocladium oryzae*; Po: *Pyricularia oryzae*; Bo: *Bipolaris oryzae*; Mo: *Microdochium oryzae*.

gens on T2 seeds. Vir et al. (1971) reported that Dithane M-45 gives complete control of seed-borne *Alternaria padwickii.*

Germination Test (In Between Paper and In-Soil)

Treatments and methods with respect to normal & abnormal seedling and dead seed significantly affected all the tested varieties. Interaction between varieties versus methods and varieties versus treatments were also significant. In the case of normal seedlings, V1, V2 and V3 were statistically similar for the in-soil test but for the in-between paper test, V1 and V3 were not statistically similar to V2 and V4 (Table 2). Among the varieties, V1 produced the higher number of normal seedlings in both methods.

In the case of abnormal seedlings, all varieties were similar in the in-soil method. For the in-between paper method, V2 and V4 had the higher abnormality. Variety V2 and V4 had the higher number of dead seed in both methods. Among the two methods, the in-soil test had the higher number of dead seed (Table 3), which may have been due to a soil problem or heterogeneous conditions in respect of moisture and depth of seed placement.

With respect to treatments, all varieties produced a statistically higher number of normal seedling, in case T1 compare to T2 and T3. Among the varieties, V1 had the higher number of normal seedling when V4 had the lowest number. In case of abnormal seedlings, T2 and T3 produced a slightly higher number compared to T1. Among the varieties, V2 and V4 produced the higher number of abnormal seedlings. All the varieties had a statistically higher number of dead seeds in the T2 and T3 treatments compared to T1 (Table 3).

The reason behind the higher percentage of abnormal and dead seeds in the T2 and T3 treatments compared with T1 may be differences in seed quality. The source of seeds for T2 and T3 were farmers' original, which contains some unfilled and immature grains. Besides, chemicals may also affect germination or normal growth of seedlings. Chemicals do not maintain the desired level during germination and do not protect germinating seeds and seedlings from seed borne and soil-borne pathogen (Misra et al., 1994a).

Rate of Germination Index

Among the varieties, V2 showed higher and V4 showed lower vigor in all treatments whereas V1 and V3 showed medium vigor, except for V1T1 (Figure 1), which showed higher vigor. Almost all the varieties showed lower RGI

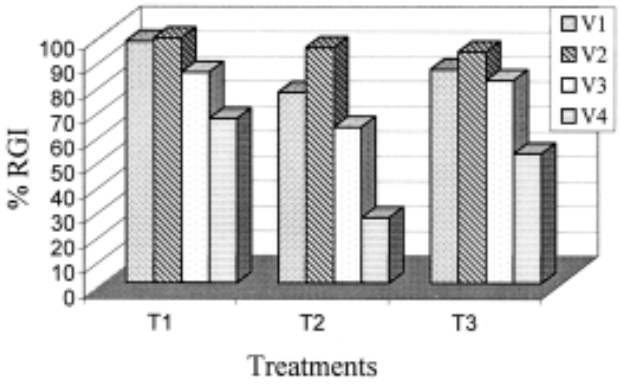

Figure 1. Means for rate of germination index (RGI) of different varieties and treatments.

Table 2. Germination status of different varieties as affected by methods.

Variety	In-soil (M2)			Between paper (M1)		
	N	Ab	D	N	Ab	D
V1	90.06 ab	1.53 b	8.42 b	95.11 a	1.53 b	3.36 c
V2	80.50 b	1.82 b	23.58 a	85.89 b	4.25 a	9.64 b
V3	81.06 b	1.53 b	17.69 b	94.78 a	2.75 b	2.44 c
V4	64.89 c	2.11 b	33.00 a	79.87 b	3.56 a	16.57 b
5% LSD	6.02	1.23	7.93	6.02	1.23	7.93

In a column, means followed by a common letter are not significantly different at the LSD 5%. N: Normal seedling; Ab: Abnormal seedling; D: Dead seed.

Table 3. Germination status of different varieties as affected by treatments.

Variety	T1			T2			T3		
	N	Ab	D	N	Ab	D	N	Ab	D
V1	96.71 a	0.71 b	2.58 c	90.29 a	2.21 ab	7.5 bc	90.75 a	1.67 b	7.58 bc
V2	88.50 b	2.87 ab	8.63 bc	82.13 bc	2.86 ab	15.29 b	78.96 c	3.38 a	25.92 a
V3	89.75 ab	1.92 ab	8.75 bc	86.88 b	1.92 ab	11.21 bc	87.16 b	2.58 ab	10.25 bc
V4	86.00 bc	2.08 ab	11.92 bc	61.89 d	3.17 ab	34.94 a	69.25 d	3.25 a	27.50 a
5% LSD	7.37	1.51	9.71	7.37	1.51	9.71	7.37	1.51	9.71

In a column, means followed by a common letter are not significantly different at the LSD 5%. T1: Best seed; T2: Treated seed; T3: Original farmers' seeds; N: Normal seedling; Ab: Abnormal seedling; D: Dead seed.

Table 4. Lethal seed infection level (%) of different varieties under different treatment levels.

Variety	Treatment	Condition	Physiologically	*Fusarium moniliforme*	*Curvularia* spp.	*Alternaria padwickii*
V1	T1	Ab	0.92	0.58	-	-
		D	0.08	0.08	-	-
	T2	Ab	1.67	-	0.17	-
		D	5.08	-	0.08	-
	T3	Ab	2.08	0.50	0.25	0.17
		D	4.83	0.92	0.25	0.33
V2	T1	Ab	3.75	0.83	0.08	0.08
		D	3.50	0.50	0.17	-
	T2	Ab	5.25	-	-	-
		D	12.33	0.08	-	-
	T3	Ab	5.33	1.08	0.25	0.25
		D	12.92	2.25	0.25	0.33
V3	T1	Ab	3.67	0.17	0.08	0.33
		D	1.67	0.15	0.08	0.67
	T2	Ab	2.33	-	-	-
		D	1.91	-	-	-
	T3	Ab	3.58	0.42	0.08	0.08
		D	3.92	1.42	0.33	1.58
V4	T1	Ab	2.0	0.67	0.08	-
		D	3.25	1.33	0.25	-
	T2	Ab	3.75	-	-	-
		D	28.08	0.25	-	-
	T3	Ab	4.58	1.42	0.33	-
		D	18.25	7.33	0.58	0.83

in T2 (chemically treated). Seed treatment, pathogens, seed quality, and genetic constitution are some of the factors influencing vigor, distinct from germinability (Seshu et al., 1988).

Lethal Seed Infection

The highest lethal seed infection caused by *Fusarium moniliforme* (7.33% dead seed & 1.42% abnormal seedlings) was observed in farmer's original seed for V4 variety followed by V2 (2.25% dead seed & 1.08% Ab. seedling) (Table 4). *Alternaria padwickii* and *Curvularia* spp. also caused lethal seed infection in farmer's original seed. Among the cultivars, V1 had the lowest infection level and among the treatments, and farmer's original seeds (T3) had the highest. Fluffy mycelia and microconidia of *Fusarium moniliforme* cover the entire seed, and infected seedlings with necrotic lesions on roots die before or after transplanting (Misra et al., 1994b). Ou (1985), reported seedling death due to *Alternaria padwickii*.

From the above study, it was found that the best seed produced the highest percent of germination, normal seedling, and high seedling vigor compared to seed treatment and farmer's original seed. The degree of infection by different pathogens was the highest in farmer's original seed. Among the varieties V1 (C-4/ Malagkit) and V2 (IR59) showed the better performance with respect to normal seedling, higher percent germination, and higher vigor. Chemical seed treatment is better for controlling fungal disease, but it was found to affect seed germination and vigor. It may, therefore, be concluded that physically good-looking seed may be used for better germination and seedling growth. Chemicals for seed treatment could be used for seed exchange and trade to eliminate fungal pathogens.

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在不同條件下評估某些品種水稻種子之健康情形

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在國際水稻研究所我們進行實驗以評估在不同處理條件下某些品種水稻種子之品質。對發芽率及病害感染率而言，品種及處理都是變異之主因。在諸品種中，C-4/Malaqkit (V1) 及 IR59 (V2) 在所有處理當中（化學處理 T2 例外）具最高之發芽率。以病害而言，*Alternaria padwickii* 乃主要病源；而其感染率在所有品種中（R30，即 V4，除外）無論是最好的種子 (T1) 或農人的原始種 (T3) 都是最高的。經化學處理之種子則無病原菌感染。在所有處理中，無論是在土壤中或在紙張之間的發芽試驗均顯示 V1 具最高比率之正常幼苗而 V4 最低。在土壤發芽試驗，所有品種種子之不正常幼苗統計上是相似的；但在紙張之間的發芽試驗則結果不同。V2 及 V4 之不正常幼苗率（紙張的發芽試驗）及死亡種子率（土壤中發芽試驗）均較高些。在所有處理中 V2 具最高之活力 (Vigor) 而 V4 則最低。在各種處理中， T1 給予任何品種最高之活力及最多之正常幼苗。最高的種子感染致死率來自 *Fusarium moniliforme*, *Alternaria padwickii* 及 *Curvularia* spp. 也可導致種子因感染而死亡。

關鍵詞：致死之種子感染；種子發芽率指數；種子健康；種子活力。