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Seed Health Testing of Recombinant Inbred Lines Derived from Cross between Kalonunia and Pusa Basmati 1637

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Detection of seed born pathogen is important as it disseminates to the next crop using seed as medium. Identification of the hidden pathogen with seed will offer the way to take precautionary measures for seed treatment. Study was carried out to evaluate the seed health, such as

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germination, pre-emergence death, post-emergence test, infection (%) and presence of different seed-born pathogen of 11 inbred lines developed from a cross between Kalonunia and Pusa Basmati 1637 along with these two parents. Four different genera of pathogens were identified, *Aspergillus, Penicillium, Curvularia* and *Magnaporthe*. *Aspergillus* was predominant and it was found in all the seed lots. As the duration of seed in storage increased the germination reduced. No notable increase or decrease in infection was noted with the duration of seed storage.

Keywords: Seed born pathogen; rice; inbred lines.

1. INTRODUCTION

Seeds are the cornerstone of agriculture, cradling the potential for bountiful harvests and a thriving food system. However, seeds can harbour unseen threats, the pathogens like fungi, bacteria, and viruses that can wreak havoc on crops. This hidden danger underscores the critical importance of seed health, a concept encompassing the absence of seed-borne diseases and pests. Diseased seeds can significantly reduce yields by estimates ranging from 15% to a staggering 90%, and in few cases 100% impacting food security and economic stability.

The spread of these pathogens through infected seeds can introduce new diseases to previously unaffected areas, jeopardizing entire agricultural ecosystems. Ensuring seed health is paramount not only for maximizing crop yields but also for safeguarding food security and environmental sustainability. In recent years seed has become an international commodity used to exchange germplasm around the world. Seed is, however, also an efficient means of introducing plant pathogens into a new area as well as providing a means of their survival from one cropping season to another [1].

Seed health testing is thus routinely carried out in most countries for domestic seed certification, quality assessment and plant quarantine [2]. Seed health testing is an integral for all seed companies in disease risk management [3]. The test used depends on the organism being tested for and the purpose of the test quality assurance or phytosanitary purposes when seed is exported [4]. Maintaining seed health is not merely about protecting individual plants; it safeguards entire agricultural ecosystems.

By prioritizing seed health, we pave the way for a more resilient agricultural future. Seed-borne fungi are one of the most significant biotic constraints on seed production worldwide. They cause both pre-emergence and post emergence death of seeds affecting seedling vigour which in result causes morphological variance in plants and reduction in germination [5,6]. Fungi outnumber all other types of pathogens that attack plants and cause a very serious economic impact on agricultural production due to their ability to induce diseases of cultivated crops that result in important yield losses [7]. The degree of seed infection is contingent upon various environmental factors, including elevated relative humidity, optimal temperature, and a high seed moisture content. Considering importance of presence of seed-born pathogen in stored seed, the present study was performed to detect the presence seed associated pathogens in different seed lots of recombinant inbred lines of rice.

2. MATERIALS AND METHODS

The experiment was conducted during August, 2023 and November, 2023 at the Department of Seed Science and Technology and the Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal.

The materials used were obtained from Niche Area of Excellence's ongoing project focused on developing rice lines with enhanced blast resistance. Eleven inbred lines were used along with one blast resistant parent and one blast susceptible (Kalonunia) parent. Agar plate is the most common method used for identification of seedborne fungi [8]. The Agar Plate Method was used to assess the presence and incidence of seed-borne fungi. Forty seeds were collected from a random sample and tested for Agar Plate Method. Water agar at 8 g/L concentration was prepared following standard protocols. The prepared water agar solution was autoclaved for sterilization at 121°C for 30 minutes at 15 psi. The sterilized agar solution was then poured into pre-sterilized 90mm (90*17mm) diameter plastic petri dishes at 30ml/plate to create a solidified growth medium in a laminar airflow cabinet to avoid contamination.

A random sample of 40 seeds were collected and each sterilized petri dish containing solidified water agar medium received 10 seeds. Four petri dishes were used for each sample, resulting in a total of 26 petri dishes. The petri dishes containing seeds were sealed and incubated at a constant temperature of 20°C ± 2°C under alternating cycles of 12 hours light and 12 hours darkness for 7 days. After incubation, the seeds were examined under a stereo binocular microscope to record the incidence of different seed borne fungi. Different fungal growth was identified based on their morphological characteristics. The number of seeds colonized by each fungal type was recorded for each petri dish. Germination of these incubated seeds were also recorded. Infection percentage was calculated as:

Infection (%) = (Infected number of seeds (pre-emergence + post-emergence))/ (Total seeds) ×100

3. RESULTS AND DISCUSSION

Rice suffers from several biotic and abiotic stresses during cultivation and seed storage resulting heavy losses to farmers and reduction seed quality during storage. Among the several constrains to rice production, diseases caused by fungi, nematodes and bacteria cause major economic loses [9,10,11]. Fungi play significant roles in reducing the quality of rice seed during infection [12].

3.1 Analysis of Variance

The analysis of variance test is the initial step in analysing factor that effect a given data set. The statistical analysis of variance of seed tested during August, 2023 and November, 2023 showed highly significant variation among the rice genotypes for seed germination, pre-emergence death, post emergence death and infection (Table 1). The statistical analysis of pooled data also showed high signification variation among the rice genotypes for all the four characters.

3.2 Seed Germination (%)

The seed germination varied from 75.00-100.00% and 70.00-100.00% with a mean of 88.85 and 84.62% during August and November, respectively (Table 2A). Maximum seed germination was reported for NAE-6, NAE-10 and NAE-12 (100.00%) during when tested during August and lowest seed germination was recorded for Kalo Nunia.

The genotype NAE-10 and NAE-12 retained 100.00% germination when they were tested during November (Table 2A). The germination (%) of the breeding lines, namely 192, 207, NAE-11 and Kalo Nunia remained the same during August and November. However, the germination fallen down for all the breeding lines when tested during November.

The mean of August and November varied from 75.00 to 100.00% with a mean of 86.73% (Table 2A). Highest mean germination was noted for NAE-10 and NAE-12 (100.00%). Lowest mean germination was recorded for Kalo Nunia and NAE-4 (75.00%). Some of the fungal pathogens are known to cause seed rot leading to decrease in seed germination, pre-emergence death and post-emergence death [13].

Table 1. ANOVA of different components For August month

Source of Variation	D.f.	Mean Sum of Square (August, 2023)			
		Germination %	% Pre emergence death	% Post Emergence Death	% Infection
Replication	1	0.000	0.000	61.538	61.538
Treatment	12	253.846***	253.846 ***	70.513.	225.000 **
Error	12	33.333	33.333	28.205	36.538
Mean Sum of Square (November, 2023)					
Replication	1	138.462	246.154 *	61.538	553.85 **
Treatments	12	221.154 ***	213.462 **	59.615 NS	211.54 *
Error	12	30.128	37.821	69.872	53.85
Mean Sum of Square (Pooled ANOVA)					
Replication	1	15.385	34.615	3.846	15.385
Treatments	12	182.532 *	192.788 **	52.885 **	184.135 **
Error	12	54.968	40.865	12.179	34.135

*Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'*

Table 2A. Seed health parameters and associated pathogens

Entries	Germination (%)			Pre-emergence death (%)			Post-emergence death (%)		
	D1	D2	Mean	D1	D2	Mean	D1	D2	Mean
182	95.00	90.00	92.50	15.00	10.00	12.50	10.00	5.00	7.50
192	80.00	80.00	80.00	20.00	20.00	20.00	0.00	0.00	0.00
205	80.00	75.00	77.50	30.00	25.00	27.50	0.00	0.00	0.00
207	90.00	90.00	87.50	10.00	0.00	5.00	10.00	20.00	15.00
NAE-1	90.00	70.00	80.00	10.00	30.00	20.00	10.00	10.00	10.00
NAE-3	90.00	85.00	87.50	10.00	15.00	12.50	0.00	0.00	0.00
NAE-4	80.00	70.00	75.00	20.00	30.00	25.00	5.00	5.00	5.00
NAE-6	100.00	90.00	95.00	0.00	10.00	5.00	15.00	10.00	12.50
NAE-10	100.00	100.00	100.00	0.00	0.00	0.00	10.00	10.00	10.00
NAE-11	95.00	95.00	95.00	10.00	5.00	7.50	0.00	0.00	0.00
NAE-12	100.00	100.00	100.00	0.00	0.00	0.00	0.00	5.00	2.50
PB 1637	95.00	80.00	87.50	5.00	20.00	12.50	10.00	0.00	5.00
Kalo Nunia	75.00	75.00	75.00	30.00	25.00	27.50	10.00	5.00	7.50
Mean	88.85	84.62	86.73	12.31	14.62	13.46	6.15	5.38	5.77
Range	75.00-100.00	70.00-100.00	75.00-100.00	0.00-30.00	0.00-30.00	0.00-27.00	0.00-15.00	0.00-20.00	0.00-15.00
LSD (5%)	11.98	13.39	15.56	11.98	15.85	13.72	NS	NS	7.33
LSD (1%)	16.70	18.68	21.70	16.70	22.10	19.14	NS	NS	10.23

3.3 Pre-Emergence Death (%)

The death of a seedling before or shortly after emergence due to decomposition of the root and/or lower stem by pathogen is known as pre-emergence death (Fig. 1). With pre-emergence death, seeds and seedlings are affected during or after germination but before emergence resulting in poor stands.

The pre-emergence death varied from 0.00 to 30.00% in both of the testing time (Table 2A). With a mean of 12.31% and 14.62%, respectively during August and November. The mean of two time of testing of respective individual varied from 0.00 to 27.00%. It was found that the pre-emergence death increased from 12.31% to 14.62% with passes of storage duration. The breeding lines NAE-10 and NAE-12 did not show any pre-emergence death during entire period of testing. Maximum pre-emergence death (27.00%) was observed for the breeding line 205 and Kalonunia. Some contradictory result was also obtained for the breeding line 207. It showed pre-emergence death of 10.00% during August, whereas no pre-emergence death was recorded during November. Kalonunia had 30.00% pre-emergence death during August which decreased to 25.00% during November.

3.4 Post-Emergence Death (%)

If seedlings emerge and then wilt and collapse, that is called post-emergence death (Fig. 1). Post-emergence death also deteriorates the crop stand. It varied from 0.00 to 15.00% and 0.00 to 20.00% during August and November testing (Table 2A). Four breeding lines, namely 192, 205, NAE-3 and NAE-11 showed no post-emergence death during entire period of testing. Highest mean of post-emergence death (15.00%) was recorded for the breeding line 207 followed by NAE-6 (12.00%), NAE-1 (10.00%) and NAE-10 (10.00%). Some cntradictory results also reported for this character. The breeding lines 182 and NAE-6 had lower post-emergence death during November as compared to August.

3.5 Infection (%)

Infection occurs when the pathogen invades the plant tissue and establishes a parasitic relationship between itself and the plant. These pathogens cause seed discolouration, seed rot, reduce seed germination, and vigour in seedlings, as well as making the plant weak during its early growth period.

The infection varied from 0.00 to 40.00% and 5.00 to 40.00% during August and November, respectively (Table 2B). No pathogen was reported for NAE-12 when tested during August, however it showed 5.00% infection when tested during November. Maximum infection mean of two testing times was observed for Kalonunia (35.00%) followed by NAE-1 (30.00%), NAE-4 (30.00%), 205 (27.00%), 182 (20.00%), 207 (30.00%) and 192 (30.00%).

3.6 Presence of Pathogens

Seed infection is dominated by fungal pathogen. Fungal pathogens that are seed-borne are comparatively challenging to manage because the fungal hyphae get established and becomes dormant. Fungi infects crops on the field and they persist and proliferate in storage resulting in increased fungal contamination with duration of seed storage [14]. Most crop diseases that are important economically are seed-borne and seed transmitted, including blast disease of rice. With the consideration that the pathogenic seed transmission plays and important role in the spread and development of epidemic diseases in rice, then the seed health tests need to be incorporated into the seed certification process. Seed health test is required to detect the presence of pathogen or seed health status.

In this study, the major fungal genera contaminating rice in order of decreasing predominance were *Aspergillus, Penicillium, Curvularia* and *Magnaporthe*. Only one seed lot had *Magnaporthe* pathogen. *Aspergillus* and *Penicillium* were the most common mycoflora [15,16,17,18,19] as found in this piece of work.

All the seed lots had the infection of seed-borne pathogen and *Aspergillus niger* (Fig. 2) was found in all the seed lots (Table 2B). *Aspergillus* is one of the dominant fungi in rice, contaminating in rice of Malaysia [20], India [21], Philippines [22] and Vietnam [23]. *Aspergillus* is most prevailing fungus in storage of rice seed reported by [8,24,19 and 25].

Penicillium was in seed lot of 182, NAE-3 and NAE-11 (Fig. 3A&B). Citrinin is considered to be the major causal agent of the yellow rice disease described for a long time in Japan. The characteristic colour in this product is due to the presence of citrinin-producing *Penicillium* species, mainly *Penicillium citreoviridae*, *Pennicillium atrium* and *Penicillium islandicum*. *Penicillium* was categorized as one of the most predominant fungi in storage of rice [8].

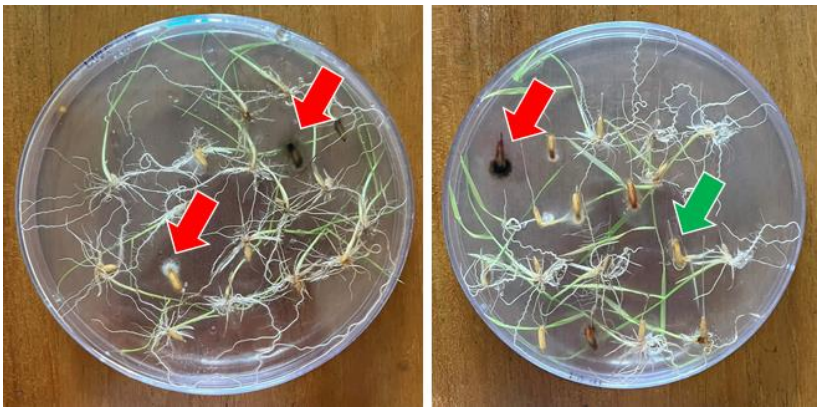

Fig. 1. Pre-emergence death shown in red arrows. Post-emergence death shown in green arrow

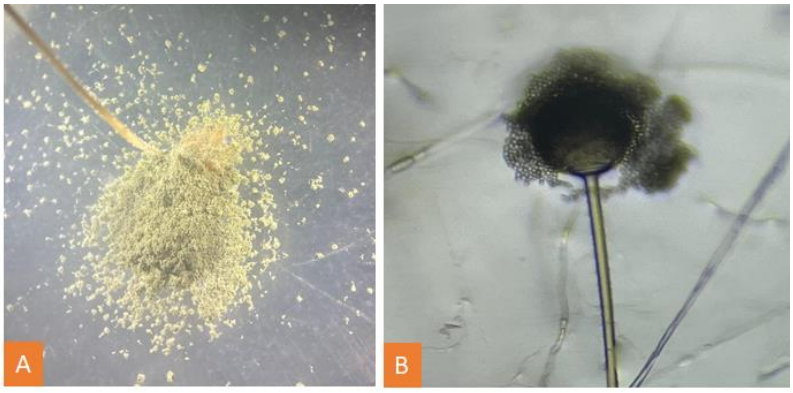

Fig. 2. *Aspergilius niger*

Table 2B. Seed health parameters and associated pathogens

	Infection (%)			Pathogen identified		
	D1	**D2**	**Mean**	**D1**	**D2**	**All Pathogen**
182	25.00	15.00	20.00	*Aspergillus, Pencillium*	*Aspergillus*	*Aspergillus, Penicillium*
192	20.00	20.00	20.00	*Aspergillus*	*Penicillium, Aspergillus*	*Penicillium, Aspergillus*
205	30.00	25.00	27.50	*Aspergillus*	*Aspergillus*	*Aspergillus*
207	20.00	20.00	20.00	*Aspergillus, Curvularia*	*Magnaporthe*	*Aspergillus, Curvularia Magnaporthe*
NAE-1	20.00	40.00	30.00	*Aspergillus*	*Aspergillus*	*Aspergillus*
NAE-3	10.00	15.00	12.50	*Aspergillus*	*Aspergillus, Penicillium*	*Aspergillus, Penicillium*
NAE-4	25.00	35.00	30.00	*Aspergillus*	*Aspergillus*	*Aspergillus*
NAE-6	15.00	20.00	17.50	*Aspergillus*	*Aspergillus*	*Aspergillus*
NAE-10	10.00	10.00	10.00	*Curvularia*	*Aspergillus*	*Curvularia, Aspergillus*
NAE-11	10.00	5.00	7.50	*Aspergillus, Curvularia*	*Aspergillus, Penicillium*	*Aspergillus, Curvularia, Penicillium*
NAE-12	0.00	5.00	2.50	*Aspergillus*	*None*	*Aspergillus*
PB 1637	15.00	20.00	17.50	*Aspergillus*	*Aspergillus*	*Aspergillus*
Kalonunia	40.00	30.00	35.00	*Aspergillus*	*Aspergillus*	*Aspergillus*
Mean	18.46	20.00	19.23	-	**-**	**-**
Range	0.00-40.00	5.00-40.00	2.50-35.00	-	**-**	**-**
LSD (1%)	13.39	NS	12.35	-	**-**	**-**
LSD (5%)	18.68	NS	17.23	-	**-**	**-**

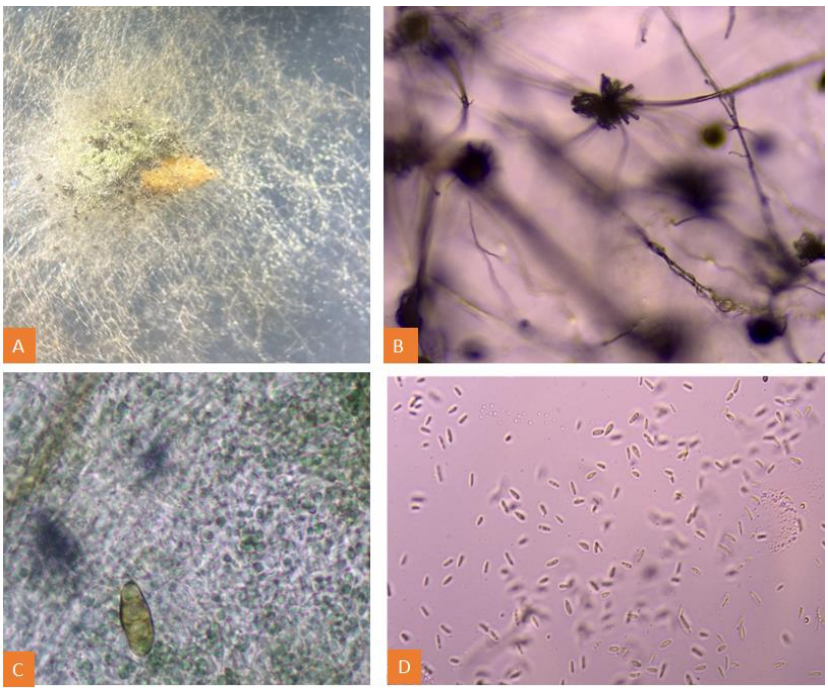

Fig. 3. A&B) Penicillium; C) *Curvularia*; D) *Pyricularia oryzae*

Curvularia was reported in 207, NAE-10 and NAE-11 (Fig. 3C). It is also known as predominant fungus in rice seed storage. Species of the genus Curvularia has been reported to infect the embryo of the seed, therefore, reducing the percentage of germination of rice seeds [9,26,27,28,29].

Only one seed lot, namely 207 has been infect with *Magnaporthe* (Fig. 3D). *Magnaporthe* (*Pyricularia oryzae*) is reported to cause very low seed infection by Martini et al. [30]. Earlier the pathogen of rice blast was known as *Pyricularia oryzae*. It is a dynamic pathogen and can adapt quickly to the conditions of the host plant. This pathogen also has a high degree of genetic diversity and the ability to produce the new breeds [31-33].

4. CONCLUSION

The experiment was conducted to appraise the presence of seed-born pathogen in 13 rice seed lots. Experiment was carried out in agar plate. Germination reduced with increase in storage duration. Pre-emergence and post-emergence death were also recorded. Four different pathogens were identified in this study, namely *Aspergillus, Penicillium, Curvularia* and *Magnaporthe*. Among these, *Aspergillus* was dominating and it was found in all the seed lots followed by *Penicillium* and *Curvularia*. This piece of study will provide the information about the presence of rice seed born pathogen under agro-climatic condition of Tarai Zone of West Bengal. However, this experiment could be carried out with a particular interval for one year on stored seed.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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