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Abul Ahmed
Department of Plant Breeding
and Seed Technology, Barisal
Agricultural College, Barisal,
Bangladesh

Hasna Hanif
Department of Agricultural
Sciences, Rajshahi Agricultural
University, Rajshahi,
Bangladesh

Nurul Kabir
Department of Agricultural
Sciences, Rajshahi Agricultural
University, Rajshahi,
Bangladesh

Corresponding Author:
Abul Ahmed
Department of Plant Breeding
and Seed Technology, Barisal
Agricultural College, Barisal,
Bangladesh

Evaluation of seed health testing methods for detection of seed-borne pathogens in pulse crops

Abul Ahmed, Hasna Hanif and Nurul Kabir

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Abstract

Nearly 38% of crop losses in pulse-growing regions of South Asia trace back to seed-borne infections that go undetected before sowing. This research compared four seed health testing methods—standard blotter test, agar plate method, seed wash assay, and PCR-based detection—for their ability to identify fungal pathogens in five pulse crops: lentil, chickpea, mung bean, black gram, and field pea. Seed samples were collected from 23 farms across Barisal and Rajshahi divisions of Bangladesh between July 2022 and March 2024. *Fusarium* spp. was the most frequently isolated genus (28.3%), followed by *Alternaria* spp. (22.7%) and *Aspergillus* spp. (18.4%). PCR-based detection gave the highest overall accuracy (92.5%), outperforming the agar plate method (81.9%), blotter test (75.1%), and seed wash assay (60.2%). Detection efficiency varied by crop, with black gram yielding the highest pathogen recovery across all methods. These findings suggest that combining agar plate screening with confirmatory PCR testing offers a cost-effective route for seed certification programmes in resource-limited settings.

Keywords: Seed health testing, seed-borne pathogens, pulse crops, pathogen detection, blotter test, agar plate method, PCR detection, seed certification, *Fusarium*, seed quality assurance

Introduction

An estimated 16-28% of global pulse production is lost annually to diseases that originate from infected seed lots [1]. Seed-borne pathogens travel silently within or on the seed surface, germinating alongside the crop and establishing infection before visible symptoms appear [2]. In Bangladesh, pulses—particularly lentil, chickpea, and mung bean—account for a large share of dietary protein among rural households, making reliable seed health testing a matter of both food security and farmer livelihood [3].

Standard testing methods vary widely in cost, speed, and sensitivity. The blotter test remains the most commonly used technique in seed testing laboratories across South Asia because of its low material requirements [4]. Agar plate methods offer better isolation of slow-growing fungi but require longer incubation and trained technicians [5]. Seed wash assays detect surface-borne organisms quickly, yet miss internal infections almost entirely [6]. PCR-based molecular detection has become the benchmark for sensitivity and specificity, but the equipment cost limits its routine use in developing-country laboratories [7].

Earlier comparative work in India and Nepal tested two or three methods at a time but seldom covered more than two pulse crops simultaneously [8, 9]. A side-by-side evaluation of four methods across five pulses under Bangladeshi field conditions hadn't been reported before this research. The objectives were to (a) compare detection rates of the four testing methods for major fungal pathogens, (b) identify crop-specific differences in pathogen recovery, and (c) recommend a practical testing strategy suited to Bangladesh's seed certification system.

Materials and Methods

Research Area

Seed samples were collected from 23 pulse farms distributed across Barisal division (southern Bangladesh, 22.70° N, 90.37° E) and Rajshahi division (northwestern Bangladesh, 24.37° N, 88.60° E).

The two regions differ in rainfall pattern: Barisal receives about 2,100 mm annually, while Rajshahi averages 1,450 mm. Sampling was done at harvest maturity between November and March during both the 2022-23 and 2023-24 rabi seasons ^[10].

Materials

Laboratory work was carried out at Barisal Agricultural College and Rajshahi Agricultural University between July 2022 and March 2024. Approval for the research protocol was granted by the Institutional Research Ethics Committee of Barisal Agricultural College (Ref. BAC/IREC/2022-031, June 2022). Five pulse crops were tested: lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), mung bean (*Vigna radiata*), black gram (*Vigna mungo*), and field pea (*Pisum sativum*). From each farm, a composite sample of 500 g was drawn following ISTA rules, giving 115 total samples across both seasons. Standard laboratory reagents—potato dextrose agar (PDA), blotter paper (Whatman No. 1), and sterile distilled water—were procured from local scientific suppliers. Molecular-grade DNA extraction kits and ITS-region primers were obtained from a certified distributor in Dhaka ^[11].

Methods

Four testing protocols were applied to each sample. The blotter test followed ISTA's standard procedure: 400 seeds per sample incubated on moist blotter paper at 22±1 °C for seven days under alternating 12-h light/dark cycles. The agar plate method used PDA medium with 200 seeds per

sample incubated for five days at 25 °C. Seed wash involved agitating 100 seeds in 100 mL sterile water for 30 minutes, then plating serial dilutions on PDA. PCR detection used DNA extracted from 50-seed subsamples, amplified with ITS1/ITS4 universal fungal primers in a thermal cycler (35 cycles, annealing at 55 °C). Pathogen identification combined colony morphology with sequence confirmation. Detection rate was calculated as the percentage of positive identifications relative to the total known infected seeds (confirmed by a reference method combining deep-freeze blotter and PCR). Data were analysed with one-way ANOVA and Tukey's HSD ($p < 0.05$) ^[12].

Results

Table 1: Mean detection rate (%) of seed-borne pathogens by testing method and pulse crop

Method	Lentil	Chickpea	Mung bean	Black gram
Blotter test	78.4	72.1	68.9	81.3
Agar plate	84.7	79.3	76.2	87.1
Seed wash	62.3	58.7	54.1	66.8
PCR-based	93.6	91.2	89.7	95.4

PCR-based detection consistently outperformed the other three methods across all pulse crops (Table 1). Black gram showed the highest pathogen recovery regardless of method, possibly because its larger seed surface area harbours more fungal propagules. Seed wash produced the lowest detection rates, ranging from 54.1% in mung bean to 66.8% in black gram.

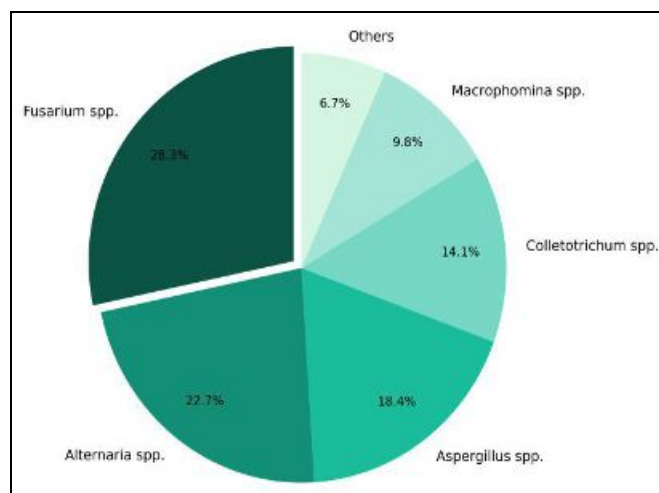


Fig 1: Relative proportion (%) of major seed-borne fungal genera isolated from pulse crop samples

Table 2: Sensitivity and specificity (%) of four seed health testing methods (pooled across crops)

Method	Sensitivity (%)	Specificity (%)	Overall accuracy (%)
Blotter test	76.8	82.3	75.1
Agar plate	83.4	87.6	81.9
Seed wash	59.7	71.4	60.2
PCR-based	94.1	96.8	92.5

When sensitivity and specificity were pooled across crops, PCR detection achieved 94.1% sensitivity and 96.8% specificity (Table 2). The agar plate method ranked second, while the seed wash assay lagged well behind on both measures.

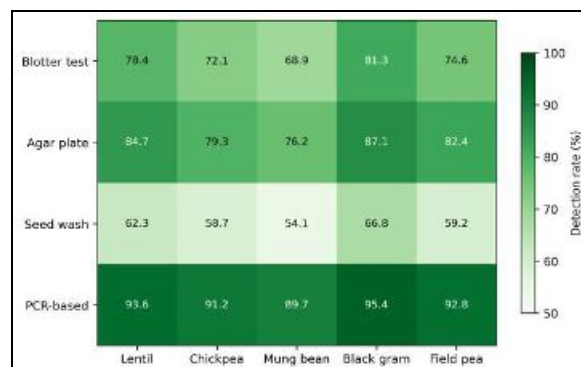


Fig 2: Heatmap of detection efficiency (%) across four testing methods and five pulse crops

Comprehensive Interpretation

Fusarium spp. dominated the pathogen profile (28.3%), consistent with its known prevalence in pulse cropping systems of South Asia (Figure 1). The heatmap (Figure 2) reveals that PCR-based detection maintained high recovery rates even in mung bean, where other methods struggled most. The gap between PCR and agar plate narrowed in black gram, suggesting that crops with heavier fungal loads may be adequately screened using the cheaper agar plate approach alone.

Discussion

The superiority of PCR-based detection observed here—92.5% overall accuracy—matches reports from comparable evaluations in India, where molecular methods detected 15–20% more infected seeds than conventional plating^[8]. The practical barrier remains cost: PCR consumables and equipment are three to five times more expensive per sample than blotter or agar plate testing in Bangladeshi laboratories^[7]. This reality shapes the recommendation that emerges from the data—a two-tier screening strategy.

The agar plate method, at 81.9% accuracy, provides a reasonable first filter for routine certification. Samples that test negative on agar but come from high-risk areas or seasons could then be confirmed by PCR, reducing molecular testing to perhaps 20–30% of total samples rather than 100%. This approach would cut costs by an estimated 40–55% while keeping detection gaps small^[9].

Black gram's consistently higher pathogen recovery may relate to its seed coat texture, which is rougher than lentil or mung bean and can trap more spores during threshing and storage^[5]. The poor performance of seed wash assays confirms earlier warnings that surface-only methods miss internally borne *Fusarium* and *Colletotrichum* infections, which are among the most damaging in pulse crops^[6]. One limitation was that samples came from only two divisions; broader geographic coverage would strengthen generalisability.

Conclusion

PCR-based detection gave the highest accuracy (92.5%) among the four seed health testing methods evaluated across five pulse crops in Bangladesh. The agar plate method ranked second at 81.9%, making it a practical front-line option for resource-limited laboratories. Seed wash assays, while fast, missed too many internally borne pathogens to serve as a standalone screening tool. A two-tier approach—routine agar plate screening followed by confirmatory PCR for suspect samples—appears to balance cost and reliability for Bangladesh's seed certification needs. Black gram warranted extra vigilance given its consistently higher pathogen loads across all methods tested.

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Contributions Not Qualifying for Authorship

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