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ORIGINAL ARTICLE

A simple method of DNA isolation from jute (*Corchorus olitorius*) seed suitable for PCR-based detection of the pathogen *Macrophomina phaseolina* (Tassi) Goid.

C. Biswas[1], P. Dey[1], S. Satpathy[1], S.K. Sarkar[1], A. Bera[2] and B.S. Mahapatra[3]

1 Crop Protection Division, Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore, Kolkata, West Bengal India
2 Central Seed Research Station for Jute and Allied Fibres (CRIJAF), Budbud, West Bengal India
3 Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore, Kolkata, West Bengal India

Significance and Impact of the Study: Stem rot caused by *Macrophomina phaseolina* is the most important disease of jute, a bast fibre crop. Seed borne infection of the pathogen is generally detected by conventional methods such as blotter method and agar plate method followed by microscopy. But, these techniques are time consuming and not sensitive. In the present investigation *M. phaseolina* was detected from jute seeds by PCR which is a rapid and reliable technique. However, high contents of mucilage and secondary metabolites in jute seed hinder DNA isolation and PCR amplification. To address these problems we developed a Miniprep which yielded a sufficient amount of good quality DNA as compared to other methods and standardized a PCR protocol which could amplify the fungal DNA present in seed. It would enable efficient PCR-based detection of *M. phaseolina* from large number of jute seed lots.

Keywords
DNA isolation, jute seed, *Macrophomina phaseolina*, PCR.

Correspondence
Chinmay Biswas, Crop Protection Division, Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore, Kolkata, West Bengal, India.
E-mail: chinmaybiswas@rediffmail.com

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Abstract

A simple method was developed for isolating DNA from jute seed, which contains high amounts of mucilage and secondary metabolites, and a PCR protocol was standardized for detecting the seedborne pathogen *Macrophomina phaseolina*. The cetyl trimethyl ammonium bromide method was modified with increased salt concentration and a simple sodium acetate treatment to extract genomic as well as fungal DNA directly from infected jute seed. The Miniprep was evaluated along with five other methods of DNA isolation in terms of yield and quality of DNA and number of PCR positive samples. The Miniprep consistently recovered high amounts of DNA with good spectral qualities at A260/A280. The DNA isolated from jute seed was found suitable for PCR amplification. *Macrophomina phaseolina* could be detected by PCR from artificially inoculated as well as naturally infected jute seeds. The limit of PCR-based detection of *M. phaseolina* in jute seed was determined to be $0{\cdot}62 \times 10^{-7}$ CFU g^{-1} seed.

Introduction

Macrophomina phaseolina (Tassi) Goid. is a dreaded pathogen and it can infect more than 500 plant species in about 100 families (Mihail and Taylor 1995). It causes stem rot disease in jute (*Corchorus olitorius* and *Corchorus capsularis*), an important bast fibre crop. The disease is prevalent in all the jute-growing areas of the world, and the pathogen attacks any part of the plant at any stage of growth. It can cause damping off, seedling blight, leaf blight, collar rot, stem rot and root rot (Biswas *et al.* 2011). Both the cultivated species of jute viz., *tossa* jute (*C. olitorius*) and white jute (*C. capsularis*) are equally affected by this disease. Average yield loss due to this disease is about 10%, but it can go up to 35–40% in case of severe infection (Roy *et al.* 2008).

Although the pathogen *M. phaseolina* by and large perpetuates in soil, it is also transmitted by seeds (Varadarajan and Patel 1946), and the seedborne inoculum presents a serious threat to the seedling establishment or may cause havoc at the later stage of crop growth. Eliminating the infected seed lots or eradicating the seedborne inoculum by chemical or biological treatment would be the best option for managing the disease. But if it is not known which seeds are infected, it would not be possible to discard them and seed treatment would become mandatory for all the seeds before sowing. Therefore, an efficient detection technique which is rapid and sensitive is essential. The conventional methods of detecting seedborne pathogens include the blotter method, agar-plate method or plating on selective media, which are followed by microscopy for identification (Neergaard 1977; Mbofung and Pryor 2010). But these techniques are time-consuming and labour intensive, require skilled personnel and are not suited for testing large number of commercial seed samples. Moreover, the low level of specificity limits the use of these methods because other fungal pathogens with similar morphological features may also grow on the selective media. But PCR-based methods are highly sensitive and specific. Due to rapid detection, a large number of samples can also be processed within a short period of time. However, the use of PCR-based detection methods in seed assays has not been much popularized as high levels of polysaccharides generally present in seeds hinder DNA extraction, and at times, the presence of PCR inhibitors also inhibits the amplification of the recovered DNA (Mbofung and Pryor 2010). Jute seeds are rich source of mucilage and various secondary metabolites, which interfere with the DNA isolation from jute seed (Khan *et al.* 2006; Kundu *et al.* 2011). Therefore, in the present investigation, we developed a Miniprep for extracting genomic DNA as well as fungal DNA directly from jute seed. We also standardized a PCR protocol, which can amplify both jute genomic DNA and fungal DNA for PCR-based detection of the seedborne pathogen *M. phaseolina* from jute seed.

Results and discussion

PCR-based assay for detection of fungal pathogens from seed requires a robust DNA isolation technique and a suitable PCR protocol. In the present investigation, we have developed a simple DNA isolation technique, which can extract sufficient quantity of good-quality fungal DNA from *M. phaseolina*-infected jute seeds and have also standardized a PCR protocol that can amplify the isolated fungal DNA.

Table 1 Comparison of different DNA isolation methods in respect of quantity and quality of DNA and PCR amplification

Method	DNA yield (μg g^{-1} fresh mass)	($A_{260/280}$)	No. of PCR positive samples/no. of samples tested
Cetyl trimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990)	110·5	2·1	8/30
Modified Dellaporta method with silica (Echevarria-Machado *et al.* 2005)	196·7	2·7	7/30
Gem-CTAB method (Rouhibakhsh *et al.* 2008)	100·9	1·2	3/30
Mucilage-free CTAB method (Ghosh *et al.* 2009)	295·7	1·9	11/30
DNeasy plant mini kit (Qiagen)	235·3	1·5	9/30
Miniprep	579·3	1·8	28/30
CD (P = 0·05)	0·92	–	–

DNA isolation from healthy, artificially and naturally infected jute seed

A Miniprep has been developed and standardized for isolating fungal DNA from *M. phaseolina*-infected jute seed. DNA was isolated from *M. phaseolina*-inoculated jute seeds, naturally infected seeds as well as healthy seeds using the Miniprep along with five other known methods.

The Miniprep tested in this study yielded high quality of mucilage-free genomic DNA from mature seeds of *C. olitorius* with absorbance ratio of 1·8 at A260/A280. Spectral analysis showed that absorbance ratios of other methods varied between 1·2 and 2·7 (Table 1). The quantity of DNA ranged from 100·9 to 579·3 μg g^{-1} in different methods. The highest quantity of DNA to the tune of 579·3 μg g^{-1} fresh seed mass was extracted using the Miniprep.

Mucilage often binds to the large numbers of different secondary metabolites present in jute seed and co-precipitates with DNA during isolation from seeds (Ghosh *et al.* 2009). But, the present method yielded large quantity of high-quality DNA. Addition of Polyvinyl pyrrolidone (PVP) to the preheated Cetyl trimethyl ammonium bromide (CTAB) buffer during homogenization absorbed the mucilages in the early stage (Ghosh *et al.* 2009; Kundu *et al.* 2011). An additional salt treatment with higher concentration of sodium chloride after initial separation helped in removal of extra mucilage from the seed tissues. A washing of partially purified DNA pellet with simple sodium acetate led to further purification by removing the secondary metabolites. The DNA isolated from

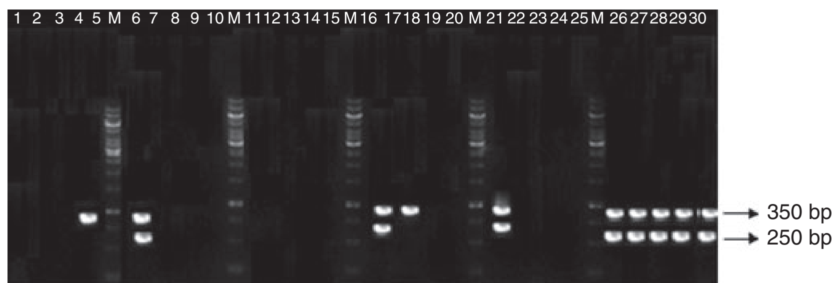

Figure 1 Comparison of different DNA isolation methods based on reproducibility of PCR amplified products from a representative batch of five *Macrophomina phaseolina*-infected jute samples. Cetyl trimethyl ammonium bromide (CTAB) method (lanes 1–5); modified Dellaporta method with silica (lanes 6–10); Gem-CTAB method (lanes 11–15); mucilage-free CTAB method (lanes 16–20); DNeasy plant minikit (lanes 21–25), present method (lanes 26–30). M-100 bp DNA ladder.

infected seed by the present method contained both jute genomic DNA and DNA of *M. phaseolina*.

PCR amplification of DNA isolated by different methods

The PCR protocol was standardized for detecting *M. phaseolina* from infected jute seed. The *M. phaseolina*-specific primer could amplify the seed DNA of infected samples generating an amplicon size of 350 bp (Fig. 1). However, the DNA isolated from infected jute seed by different methods showed differential response. In the present method, of 30 inoculated samples tested, 28 were found PCR positive (Table 1). But in case of other methods, the number of PCR-positive samples varied from 3 to 11 of 30 samples tested. Thus, it was necessary to develop a method for recovery of fungal DNA from jute seed, while eliminating the mucilage and the secondary metabolites which hinder DNA isolation as well as PCR amplification. The method described in this study has been so standardized that in addition to the fungal DNA it also amplified jute DNA generating an amplicon of 250 bp (Fig. 1).

Validation of present method for PCR-based detection of *Macrophomina phaseolina*

The present method was validated for PCR-based detection of *M. phaseolina* by testing the artificially inoculated jute seed as well as the naturally infected field samples of different cultivars. DNA was isolated from *M. phaseolina* inoculated as well as naturally infected jute seeds of three cultivars viz., JRO 204, JRO 524 and JRO 8432 following the present method. PCR amplification with *M. phaseolina*-specific primer generated an amplicon of 350 bp in cases of all the positive samples (Fig. 2). The pathogen could be detected by PCR from all the inoculated seed samples of each of the three cultivars. Although field samples were collected from plants that were assessed visually for the disease, all the seed samples did not show the presence of *M. phaseolina* in PCR.

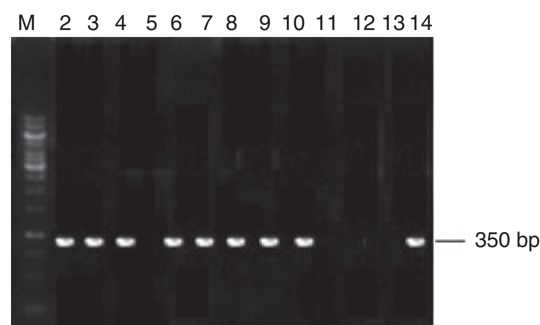

Figure 2 PCR amplification of *Macrophomina phaseolina* DNA isolated by the present method from naturally infected as well as artificially inoculated jute seeds. Lane M:100 bp DNA ladder, lanes 2–4: *M. phaseolina*-inoculated seeds of cultivars JRO 204, JRO 524 and JRO 8432 respectively; lanes 5–10: field collection of diseased samples, lanes 5–6: JRO 204, 7–8: JRO 524, 9–10: JRO 8432; lane 11–13: healthy check of JRO 204, JRO 524 JRO 8432 respectively; lane 14: *M. phaseolina* culture.

Figure 3 PCR-based detection limit of *Macrophomina phaseolina* in jute seed. Lane M: 100 bp, DNA ladder, lane 2: seed inoculated with *M. phaseolina* mycelial suspension @ $6{\cdot}2 \times 10^3$ CFU g^{-1} seed, lanes 3–15: 10^{-1} to 10^{-13} dilutions respectively; lane 16: *M. phaseolina* culture.

Detection limit of *Macrophomina phaseolina*

Known quantity of jute seeds were inoculated with mycelial suspensions of different dilutions for determining the detection limit of *M. phaseolina* in jute seed. The number of CFU present in the stock suspension was to the tune of $6{\cdot}2 \times 10^3$ CFU ml^{-1}, and *M. phaseolina* could be detected by PCR from jute seeds inoculated with mycelial suspension till 10^{-11} dilution (Fig. 3), and thus, the limit was theoretically $0{\cdot}62 \times 10^{-7}$ CFU g^{-1} seed.

The present work would enable PCR-based detection of the seedborne pathogen *M. phaseolina* from jute seeds. PCR-based detection is a rapid and effective technique for microbial diagnostics (Yamamoto 2002). It is widely used for detection of plant pathogens. In case of detecting seedborne inoculum, it is very important because it is highly sensitive. Various plant pathogenic fungi have been detected from seeds of different crops viz., carrot, lettuce etc. using PCR (Pryor and Gilbertson 2001; Mbofung and Pryor 2010). Here, we report PCR-based detection of the seedborne pathogen *M. phaseolina* from jute seed. Efficient detection of the pathogen from artificially inoculated as well as naturally infected jute seed of different cultivars demonstrates the soundness and applicability of the present method. To the best of our knowledge, it is the first report of PCR-based detection of *M. phaseolina* from jute seed. This PCR-based assay has the potential to be used for routine testing of jute seed lots for *M. phaseolina.*

Materials and methods

Fungal culture maintenance and DNA isolation

The pathogen *M. phaseolina* was isolated from infected jute (*C. olitorius*) plant (cultivar JRO 524) at the research farm of Central Research Institute for Jute and Allied Fibres, Barrackpore, Kolkata, India. The fungus culture was maintained at 25°C on potato dextrose agar (PDA). For isolation of DNA from *M. phaseolina*, a 5-mm disc of 2-day-old culture was grown for 7 days at 25 ± 1°C in 250-ml conical flask containing 50-ml potato dextrose broth (pH 5·5). The mycelia were filtered through Whatman No.1 filter paper, and DNA was isolated using the CTAB method following Biswas *et al.* 2012.

Seed inoculation

Seeds of different cultivars viz., JRO 524, JRO 8432 and JRO 204 used for artificial inoculation were collected from Crop Improvement Division, Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore, Kolkata. The naturally infected seed was collected from plants showing symptoms in an experimental set-up on the CRIJAF research farm. Jute seeds were inoculated with mycelial suspension of *M. phaseolina.* The mycelia were harvested by scraping the surface of 5-day-old fungal culture with a sterile camel hair brush into a 100-ml glass beaker containing 50-ml sterile distilled water. The mycelial suspension was prepared by mixing the suspension with a magnetic stirrer for 5 min, which contained $6{\cdot}2 \times 10^3$ colony-forming units (CFU) per ml. One gram of seed was soaked overnight in 1 ml of mycelial suspension. The CFU count per ml was determined by spreading plate technique. One millilitre of mycelial suspension was poured and spread on 10 PDA plates with 100 μl in each plate. Then the plates were incubated at 27°C for 2 days, and the colonies were counted. It was replicated thrice, and the mean value was considered. Before inoculation, the seeds were surface sterilized by treating with 0·5% sodium hypochlorite for 2 min and then with 70% ethyl alcohol for 2 min. The seeds were then washed with sterile distilled water.

DNA isolation from healthy, artificially and naturally infected jute seed

To find a suitable DNA isolation method, DNA was extracted from 30 *M. phaseolina* inoculated seed samples (cultivar, JRO 524) using each of the following methods viz., (i) CTAB method (Doyle and Doyle 1990) (ii) Modified Dellaporta method with silica (Echevarria-Machado *et al.* 2005) (iii) Gem-CTAB method (Rouhibakhsh *et al.* 2008) (iv) Mucilage-free CTAB method (Ghosh *et al.* 2009) (v) Extraction using DNeasy plant mini kit (Qiagen, GmbH, Hilden, Germany) following the recommendations of the manufacturer and (vi) Present method or Miniprep (improved salt conc. and simple sodium acetate CTAB method).

From each of the DNA samples isolated by the abovesaid methods, 2 μl of DNA was taken and diluted 1 : 50 in ultrapure water. Absorbance was recorded thrice at 260 nm as well as at 280 nm in a UV spectrophotometer (BioPhotometer; Eppendorf AG, Hamburg, Germany). DNA concentration was measured at 260 nm, and DNA yield was calculated by multiplying the mean concentration and hydration volume.

DNA was isolated by the present method (Miniprep) for its validation from four inoculated seed samples, four naturally infected samples and four healthy samples from each of the three cultivars namely JRO 524, JRO 204 and JRO 8432. Each sample for DNA isolation contained 100 mg of seeds.

Fresh dry seed was ground in preheated (60°C) freshly prepared 800 μl modified CTAB buffer (0·1 mol l^{-1} Tris-HCl, pH 9·5), 20 mmol l^{-1} EDTA (pH 8), 3 mol l^{-1}

NaCl, CTAB (10%, w/v), β-mercaptoethanol (1%, v/v) (added to the buffer just before use). PVP 3% w/v was added to the buffer so that mucilage and secondary metabolites are absorbed. The homogenate was immediately transferred to 1·5-ml Eppendorf microcentrifuge tube and was gently vortexed. Then it was incubated at 65°C for 45 min in a water bath. An equal volume of phenol/chloroform/isoamyl alcohol (25 : 24 : 1) was added to the tubes (gently mixed) and were centrifuged at 6000 ***g*** for 15 min. The aqueous phase was transferred into a 1·5-ml microcentrifuge tube and an additional 500 μl of 2 mol l^{-1} NaCl was added. Then, it was kept for half an hour at room temperature and an equal volume of absolute ice-cold isopropanol was added. The mixture was centrifuged at 7000 ***g*** for 10 min. The supernatant was discarded, and the pellet was washed with 80% (w/v) ethanol. For further purification, DNA pellet was washed by adding 1/10th volume of 3 mol l^{-1} sodium acetate (pH 5·2) and centrifuged at 6000 ***g*** for 5 min. After washing with sodium acetate 2·5 volume of ice-cold 70% (w/v) ethanol was added. It was then centrifuged at 5000 ***g*** for 5 min. The pellet was air-dried for 1 h at room temperature and then dissolved in 50 μl TE buffer (10 m mol l^{-1} Tris-HCl buffer (pH 8·0), 1 mmol l^{-1} EDTA (pH 8·0). RNase A [2 μl (5 μg μl^{-1})] and 2 μl of proteinase K (5 μg μl^{-1}) was added to the mixture and was incubated at 37°C for 1 h to eliminate RNA and protein contamination. The DNA samples were stored at −20°C until further use.

In the present method, comparatively higher concentration (10%) of preheated CTAB was used for greater efficiency of the buffer. In contrast to 1·4 mol l^{-1} NaCl in CTAB method 3 mol l^{-1} NaCl was used in buffer and an additional 500 μl of 3 mol l^{-1} NaCl was added to the supernatant after initial separation. Longer incubation (45 min) at comparatively higher temperature (65°C) was given before adding equal volume of phenol/chloroform/isoamyl alcohol (25 : 24 : 1) for removing organic contaminants. In other methods only one 70% ethanol wash was given, whereas in the Miniprep two additional steps of washing viz., 80% (w/v) ethanol wash and 3 mol l^{-1} sodium acetate (pH 5·2) wash were used. High conc. of RNase A [2 μl (5 μg μl^{-1})] and 2 μl of proteinase K (5 μg μl^{-1}) treatment were employed for the long incubation (1 h) at 37°C to remove RNA and protein contaminations, whereas Mucilage-free CTAB method used only RNaseA treatment.

Detection limit of *M. phaseolina*

From the mycelial stock suspension (6·2 × 10^3 CFU per ml) 13 serial dilutions (10^{-1} to 10^{-13}) were prepared by adding requisite quantity of sterile distilled water. Seeds of the most popular cultivar JRO 524 were inoculated with these suspensions of different dilutions separately by soaking overnight. One gram of seed was soaked overnight in 1 ml of mycelial suspension of concerned dilution. From each dilution, three seed samples (100 mg each) were used for DNA extraction followed by PCR amplification with *M. phaseolina*-specific primer.

PCR amplification

DNA samples isolated from inoculated jute seeds as well as from naturally infected seeds were assessed by PCR using *M. phaseolina*-specific primer pair MpKFI (5′-CCG CCAGAGGACTATCAAAC-3′) and MpKRI (5′CGTCCGA AGCGAGGTGTATT-3′) (Babu *et al.* 2007). A jute-specific SSR primer pair (MJM -561F5′ AGTGCAAACACGA GAGCAAAT-3′ R-5′- ATGGCATCCTCTCATCTTCCT 3′) (Mir *et al.* 2009) was also used to check the amplification of jute genomic DNA, while comparing different DNA isolation methods. Duplex PCR assay was conducted to accentuate the efficiency of current protocol. The volume of PCR was 25 μl, which contained 10× expand high-fidelity buffer (10 mmol l^{-1} Tris–HCl, 50 mmol l^{-1} KCl, pH 8·3) (Biolab, New England Biolabs, MA, USA), 25 mmol l^{-1} $MgCl_2$ (Biolab), 200 mmol l^{-1} of each dNTP (Biolab), 0·5 μmol l^{-1} of each primer (forward and reverse primers of both the primer pairs viz., *M. phaseolina* specific primer and jute SSR primer), 10 ng μl^{-1} genomic DNA and 5 U μl^{-1} Taq DNA polymerase. The amplification was carried out using a gradient thermal cycler (Bio-Rad, CA, USA). The amplification profile was 5-min initial denaturation at 94°C, denaturation at 94°C for 1 min, annealing at 56°C for 60 s and elongation at 72°C for 45 s followed by 30 cycles and a final elongation at 72°C for 7 min. PCR products were visualized in 1·6% agarose gel stained with ethidium bromide. However, in determining the detection limit of *M. phaseolina* and in validation of the present method, wherein the pathogen was detected from artificially inoculated as well as naturally infected jute seed single reaction PCR was conducted using only the *M. phaseolina* specific primer. PCR conditions and the reaction profile remained the same.

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References

Babu, B.K., Saxena, A.K., Srivastava, A.K. and Arora, D.K. (2007) Identification and detection of *Macrophomina*

phaseolina by using species specific oligonucleotide primers and probe. *Mycologia* **99**, 797–803.

Biswas, C., Sarkar, S.K. and De, R.K. (2011) Diseases of jute and mesta: present status and management options. In *Sustainable Disease Management of Agricultural Crops* ed. Biswas, S.K. and Singh, S.R. pp. 62–79. New Delhi: Daya Publishing House.

Biswas, C., Dey, P., Satpathy, S. and Satya, P. (2012) Establishment of the fungal entomopathogen *Beauveria bassiana* as a season long endophyte in jute (*Corchorus olitorius*) and its rapid detection using SCAR marker. *Biocontrol* **57**, 565–571.

Doyle, J.J. and Doyle, J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15.

Echevarria-Machado, I., Lucila, A., Sanchez, C., Hernandez-Zepeda, C., Rivera-Madrid, R. and Moreno-velenzuela, O. A. (2005) A simple and efficient method for isolation of DNA in high mucilaginous plant tissues. *Mol Biotechnol* **31**, 129–135.

Ghosh, R., Paul, S., Ghosh, S.K. and Roy, A. (2009) An improved method of DNA isolation suitable for PCR-based detection of begomoviruses from jute and other mucilaginous plants. *J Virol Methods* **159**, 34–39.

Khan, M.S.Y., Bano, S., Javed, K. and Mueed, M.A. (2006) A comprehensive review on the chemistry and pharmacology of *Corchorus* species- a source of cardiac glycosides, triterpenoids, ionones, flavonoids, coumarins, steroids and some other compounds. *J Sci Ind Res* **65**, 283–298.

Kundu, A., Sarkar, D., Bhattacharjee, A., Topdar, N., Sinha, M.K. and Mahapatra, B.S. (2011) A simple ethanol wash of the tissue homogenates recovers high quality genomic DNA from *Corchorus* species characterized by highly acidic and proteinaceous mucilages. *Electron J Biotecnol* **14**, doi: 10.2225/vol14-issue1-fulltext-4.

Mbofung, G.C.Y. and Pryor, B.M. (2010) A PCR-based assay for detection of *Fusarium oxysporum* f. sp. *lactucae* in lettuce seed. *Plant Dis* **94**, 860–866.

Mihail, J.D. and Taylor, S.J. (1995) Interpreting variability among isolates of *Macrophomina phaseolina* in pathogenicity, pycnidium production, and chlorate utilization. *Can J Bot* **73**, 1596–1603.

Mir, R.R., Banerjee, S., Das, M., Gupta, V., Tyagi, A.K., Sinha, M.K., Balyan, H.S. and Gupta, V. (2009) Development and characterization of large-scale simple sequence repeats in jute. *Crop Sci* **49**, 1687–1694.

Neergaard, P. (1977) *Seed Pathology*, Vol. **I**. New York: Halsted Press.

Pryor, B.M. and Gilbertson, R.L. (2001) A PCR-based assay for detection of *Alternaria radicina* on carrot seed. *Plant Dis* **85**, 18–23.

Rouhibakhsh, A., Priya, J., Periasamy, M., Haq, Q.M.I. and Malathi, V.G. (2008) An improved DNA isolation method and PCR protocol for efficient detection of multicomponents of begomovirus in legumes. *J Virol Methods* **147**, 37–42.

Roy, A., De, R.K. and Ghosh, S.K. (2008) Diseases of bast fibre crops and their management. In *Jute and Allied Fibre Updates* ed. Karmakar, P.G., Hazra, S.K., Ramasubramanian, T., Mandal, R.K., Sinha, M.K. and Sen, H.S. pp. 217–241. Kolkata: Central Research Institute for Jute and Allied Fibres.

Varadarajan, B.S. and Patel, J.S. (1946) Seed transmission of stem rot of jute and its control. *Indian J Agric Sci* **16**, 193–203.

Yamamoto, Y. (2002) PCR in diagnosis of infection: detection of bacteria in cerebrospinal fluids. *Clin Diagn Lab Immunol* **9**, 508–514.

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