Fungal Pathogens on Bast Fiber Crops

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Bast fiber crops are an important group of economic crops for the purpose of harvesting fibers from stems. These fibers are sclerenchyma fibers associated with the phloem of plants. They arise either with primary tissues from the apical meristem, or with secondary tissues produced by the lateral meristem. Fungal diseases have become an important factor limiting their yield and quality, causing devastating consequences for the production of bast fiber crops in many parts of the world.

bast fiber crops | molecular identification | fungal disease | DNA barcode | PCR assay

1. Introduction

Plant infectious diseases are among the most important constraints on the quality and yield of crops. It is estimated that plant diseases cause losses of 10%–15% of the world's major crops, with direct economic losses of up to hundreds of billions of dollars each year. About 70%–80% of crop diseases are caused by fungal pathogens and the damage can be very serious, significantly reducing the yield and quality of many staple food crops and economic crops like fruits, vegetables, and fiber crops [1]. In addition, several fungal pathogens can secrete a variety of toxins and metabolites harmful to humans and animals, posing a great threat to the safety of agricultural products [2]. At present, most control measures against plant fungal pathogens rely on the applications of broad-spectrum fungicides. However, such fungicides not only increase production costs, but also can bring problems such as environmental pollution, fungicide resistance, and persistent residues on foods and other consumer goods with further implications for human health. In order to minimize the damage to crops caused by fungal diseases, as well as to maximize productivity and ensure agricultural sustainability, early detection and quantification of fungal pathogens is essential for disease prevention and control. However, conventional protocols based on morphological and physiological methods are time-consuming, require significant experience, and may not be sensitive and specific for individual pathogens [3]. Moreover, many fungal pathogens can remain latent in "sub-infection" stages with no obvious symptoms and/or in low numbers, making them difficult to detect, and causing confusion with regard to their roles in diseases. These issues can contribute to delayed or wrong control measures.

During the last three decades, to overcome these problems and minimize crop losses caused by fungal diseases, a diversity of DNA molecule-based tools has been developed for the detection and identification of fungal pathogens. These techniques include conventional polymerase chain reaction (PCR) [4], quantitative PCR (qPCR) [5][6], immunocapture-PCR (IC-PCR) [7][8], droplet digital PCR (dd-PCR) [9], loop-mediated isothermal amplification (LAMP) [10], multiplex tandem PCR [11], fluorescence in situ hybridization (FISH) [12], and DNA microarrays [3]. These methods are typically faster and more accurate than those based on colony morphology, microscopic

features, and/or physiological/biochemical characters of pure fungal cultures. Indeed, methods targeting DNA sequences have been applied to detect pathogens during crops' growth, harvest and post-harvest processing stages [13]. Moreover, they have also enabled a deeper understanding of microbial populations and communities associated with crops, especially the microorganisms that are difficult or impossible to cultivate in the lab. Together, technological advances and developments in DNA molecule-based methods have allowed fast and accurate detection and quantification of several fungal pathogens simultaneously in many important crops [14][15]. Information resulting from such work has been used to improve disease control and prevention with more rational decisions about the choice of fungicides to use, the appropriate cultivar(s) to plant, and necessary sanitary measures to apply during various stages of the crop production and processing cycle [16][17][18][19].

2. Bast Fiber Crops

Bast fiber crops are an important group of economic crops for the purpose of harvesting fibers from stems [20]. These fibers are sclerenchyma fibers associated with the phloem of plants. They arise either with primary tissues from the apical meristem, or with secondary tissues produced by the lateral meristem. Bast fiber is one of four major types of natural plant fibers, with the other three being leaf fiber (e.g., banana and pineapple fibers), fruit and seed fiber (e.g., cotton and coconut fiber), and stalk fiber (e.g., straw fiber from rice, wheat, and bamboo). Bast fiber crops comprise six main species (flax, hemp, ramie, kenaf, jute, and sunn hemp) that are broadly cultivated (**Table 1**) as well as a few others (kudzu, linden, milkweed, nettle, okra, and paper mulberry) with more limited fiber production [21]. **Table 1** summarizes the main bast fiber crops, including their geographic distributions, habitats, commercial use, and main fungal diseases.

Table 1. Major types of bast fiber crops and their distributions around the world [20][21][22].

Crop	Main Distribution	Main Characters of Growth Habitat	Main Applications	Main Fungal Diseases
Flax (*Linum usitatissimum* Linnaeus)	France, Russia, Netherlands, Belarus, Belgium, Canada, Kazakhstan, China, India	Well-drained loam and cool, moist, temperate climates	Linen, flax yarn, flax seed, linseed oil	flax wilt, flax blight, flax anthracnose
Hemp (*Cannabis sativa* Linnaeus)	China, Canada, USA, Europe, East Asia, Nepal	Grows at 16–27 °C, sufficient rain at the first six weeks of growth, short day length.	Textiles, hempseed oil, prescription drugs	hemp powdery mildew, hemp leaf spot disease, hemp blight, hemp root and

Crop	Main Distribution	Main Characters of Growth Habitat	Main Applications	Main Fungal Diseases
				crown rot wilt, hemp charcoal rot
Jute (*Corchorus capsularis* Linnaeus)	India, Bangladesh, Burma, China	Tropical lowland areas, humidity of 60% to 90%, rain-fed crop	Textiles, medicine	jute anthracnose, jute brown wilt, jute leaf spot
Kenaf (*Hibiscus cannabinus* Linnaeus)	India, Bangladesh, China, Malaysia, Thailand	Sandy loam and warm, humid subtropical, or tropical climates, few heavy rains or strong winds, at least 12 h light each day	Textiles	kenaf anthracnose, kenaf lack rot, kenaf sooty mold
Ramie (*Boehmeria nivea* Linnaeus) Gaudich	China, Brazil, Philippines, India, Vietnam, Laos, Cambodia	Sandy soil and warm, wet climates, rainfall averaging at least 75 to 130 mm per month	Textiles, soil and water conservation, medicine	ramie anthracnose, ramie powdery mildew, ramie black leaf spot, ramie blight
Sunn Hemp (*Crotalaria juncea* Linnaeus)	India, USA, China	Wide variety of soil condition, altitude from 100 to 1000 m, temperatures above 28 °C, photoperiod-sensitive	Cover crop or green manure, forage producer	sunn hemp fusarium wilt, sunn hemp root rot, sunn hemp powdery mildew

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3. Fungal Pathogens of Bast Fiber Crops

As shown in **Table 1**, most bast fiber crops can grow in a diversity of geographic regions and ecological niches. However, some of them have relatively limited geographic and/or ecological distributions and can't grow well in certain environments. As a result, the types of land used to cultivate certain bast fiber crops may be limited and the

same fields may be used to grow the same crop over many years. Even for bast fiber crops with broad ecological adaptability, the limited agricultural land in certain regions and the drive to seek high commercial benefits often mean that only certain types of fields are used for growing each specific crop. In these fields, fungal infectious diseases often increase over time, leading to large yield loss, or even total destruction of the harvest. Fungal pathogens occurring on bast fiber crops are taxonomically very broad (**Table 2**).

Table 2. List of fungal pathogens on bast fiber crops identified using molecular method.

Pathogen	Disease	Method	Marker	Host Plant	Geographic Region(s)	Reference
Alternaria						
A. alternata	Hemp leaf spot	Conventional PCR	ITS	*Cannabis sativa*	Shanxi, China	[23]
A. alternata	Ramie black leaf spot	Conventional PCR	ITS, GAPDH	*Boehmeria nivea*	Hunan, Hubei, China	[24]
Cercospora						
Cercospora cf. flagellaris	Hemp leaf spot disease	Not mentioned	ITS, EF-1α, CAL, H3, actin	*Cannabis sativa*	Kentucky, USA	[25]
Colletotrichum						
C. corchorum capsularis	Jute anthracnose	Conventional PCR	ACT, TUB2, CAL, GAPDH, GS, and ITS	*Corchorus capsularis L.*	Zhejiang, Fujian, Guangxi, and Henan, China	[26]

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Pathogen	Disease	Method	Marker	Host Plant	Geographic Region(s)	Reference
C. fructicola	Jute anthracnose	Conventional PCR	ACT, TUB2, CAL, GAPDH, GS, and ITS	*Corchorus capsularis L.*	Zhejiang, Fujian, Guangxi, and Henan, China	[27]
C. fructicola	Jute anthracnose	Conventional PCR	ACT, TUB2, CAL, GAPDH, GS, and ITS	*Corchorus capsularis L.*	Zhejiang, Fujian, Guangxi, and Henan, China	[26]
C. gloeosporioides	Ramie anthracnose	Conventional PCR	ITS	*Boehmeria nivea*	HuBei, HuNan, JiangXi, and SiChuan, China	[28]
C. higginsianum	Ramie anthracnose	Conventional PCR	ITS	*Boehmeria nivea*	HuBei, China	[29]
C. phormii	New Zealand flax anthracnose	Conventional PCR	ITS	*Phormium tenax*	California, USA	[30]
C. phormii	New Zealand flax anthracnose	Conventional PCR	ITS	*Phormium tenax*	Perth, Australia	[31]
C. siamense	Jute anthracnose	Conventional PCR	ACT, TUB2, CAL,	*Corchorus capsularis L.*	Zhejiang, Fujian,	[27]

anthracnose on Boehmeria nivea caused by Colletotrichum higginsianum in China. Plant Dis. 2011, 95, 1318.

Pathogen	Disease	Method	Marker	Host Plant	Geographic Region(s)	Reference
			GAPDH, GS, and ITS		Guangxi, and Henan, China	
Colletotrichum sp.	Kenaf anthracnose	Conventional PCR	ITS	*Corchorus olitorius*	South Korea	[32]
Curvularia						
C. cymbopogonis	Hemp leaf spot	Conventional PCR	25S	*Cannabis sativa*	USA	[33]
Exserohilum						
E. rostratum	Hemp floral blight	Not mentioned	ITS, RPB2	*Cannabis sativa*	North Carolina, USA	[34]
Fusarium						
F. oxysporum	Hemp roots and crown rot	Conventional PCR	ITS, EF-1α	*Cannabis sativa*	Canada	[35]
F. oxysporum	Jute brown wilt	Conventional PCR	ITS	*Corchorus olitorius*	Dhaka, Manikgonj, Kishorgonj, Rangpur, and Monirampur, Bangladesh	[36]
F. oxysporum	Hemp wilt	Conventional PCR	ITS, EF-1α	*Cannabis sativa*	California, USA	[37]

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Pathogen	Disease	Method	Marker	Host Plant	Geographic Region(s)	Reference
F. solani	Hemp crown root	Conventional PCR	ITS, EF-1α	*Cannabis sativa*	Canada	[35]
F. solani	Hemp wilt	Conventional PCR	ITS, EF-1α	*Cannabis sativa*	California, USA	[37]
F. solani	Sunn hemp root rot and wilt	Conventional PCR	ITS, EF-1α	*Crotalaria juncea*	Ceará, Brazil	[38]
F. brachygibbosum	Hemp wilt	Conventional PCR	ITS, EF-1α	*Cannabis sativa*	California, USA	[37]
F. udum f. sp. crotalariae	Sunn hemp fusarium wilt	Conventional PCR	EF-1α, β-tubulin	*Crotalaria juncea*	Tainan, China	[39]
Glomus						
G. mosseae	Hemp root rot	Conventional PCR	25S	*Cannabis sativa*	USA	[33]
Golovinomyces						
G. spadiceus	Hemp powdery mildew	Not mentioned	ITS, 28S	*Cannabis sativa*	Kentucky, USA	[40]
G. cichoracearum sensu lato	Hemp powdery mildew	Conventional PCR	ITS	*Cannabis sativa*	Atlantic Canada and	[41]

Chinese)

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Pathogen	Disease	Method	Marker	Host Plant	Geographic Region(s)	Reference
					British Columbia.	
G. cichoracearum	*Sunn hemp powdery mildew*	Not mentioned	ITS	*Crotalaria juncea*	Florida, USA	[42]
Lasiodiplodia						
L. theobromae	Kenaf black rot	Conventional PCR	ITS	*Corchorus olitorius*	Kangar Perlis, Malaysia	[43]
Leptoxyphium						
L. kurandae	Kenaf sooty mould	Conventional PCR	ITS	*Corchorus olitorius*	Iksan, Korea	[44]
Macrophomina						
Macrophomina phaseolina	Hemp charcoal rot	Conventional PCR	EF-1α, CAL	*Cannabis sativa*	Southern Spain	[45]
Micropeltopsis						
Micropeltopsis cannabis	Unknown	Conventional PCR	25S	*Cannabis sativa*	USA	[33]
Orbilia						

Pathogen	Disease	Method	Marker	Host Plant	Geographic Region(s)	Reference
Orbilia luteola	Unknown	Conventional PCR	25S	*Cannabis sativa*	USA	[33]
Pestalotiopsis						
Pestalotiopsissp.	Hemp spot blight	Conventional PCR	25S	*Cannabis sativa*	USA	[33]
Podosphaera						
P. xanthii	Ramie powdery mildew	Conventional PCR	ITS	*Boehmeria nivea*	Naju, Korea	[46]
Pythium						
P. dissotocum	Browning and a reduction in root mass, stunting	Conventional PCR	ITS, EF-1α	*Cannabis sativa*	Canada	[35]
P. myriotylum	Browning and a reduction in root mass, stunting	Conventional PCR	ITS, EF-1α	*Cannabis sativa*	Canada	[35]
P. myriotylum	Hemp root rot and Wilt	Conventional PCR	ITS, COI, COII	*Cannabis sativa*	Connecticut, USA	[47]
P. aphanidermatum	Hemp root rot and crown	Conventional PCR	ITS	*Cannabis sativa*	California, USA	[37]

Pathogen	Disease	Method	Marker	Host Plant	Geographic Region(s)	Reference
	wilt					
P. aphanidermatum	Hemp crown and root Rot	Conventional PCR	ITS	*Cannabis sativa*	Indiana, USA	[48]
P. ultimum	Hemp crown and root Rot	Conventional PCR	ITS	*Cannabis sativa*	Indiana, USA	[49]
Rhizoctonia						
Binucleate R. spp.	Hemp wilt	Conventional PCR	25S	*Cannabis sativa*	USA	[33]
Sclerotinia						
Sclerotinia minor	Hemp crown rot	Conventional PCR	ITS	*Cannabis sativa*	San Benito County, Canada	[50]
Sphaerotheca						
S. macularis	Hemp powdery mildew	Conventional PCR	25S	*Cannabis sativa*	USA	[33]
Verticillium						
V. dahliae	flax wilt	Conventional PCR	ITS	*Linum usitatissimum*	La Haye Aubrée, France	[51]

Pathogen	Disease	Method	Marker	Host Plant	Geographic Region(s)	Reference
V. dahliae	flax wilt	qPCR	ITS	*Linum usitatissimum*	Normandy, France	[52]
V. dahliae	flax wilt	qPCR	ß-tubulin	*Linum usitatissimum*	Germany	[53]
V. tricorpus	flax wilt	qPCR	ITS	*Linum usitatissimum*	Germany	[53]
V. longisporum	flax wilt	qPCR	ß-tubulin	*Linum usitatissimum*	Germany	[53]

qPCR: quantitative PCR, ITS: internal transcribed spacer, GAPDH: glyceraldehydes-3-phosphate dehydrogenase, GS: glutamate synthetase, EF-1α: translation elongation factor 1-α, CAL: calmodulin, H3: histone subunit 3, ACT: actin, TUB2: ß-tubulin, RPB2: RNA polymerase subunit B2, COI: cytochrome oxidase subunit I, COII: cytochrome oxidase subunit II.

4. Development of Molecular Identification of Bast Fiber Fungal Pathogens

At present, most diagnosis of bast fiber diseases rely on disease symptoms and, when available, cultural characteristics of isolated fungal pathogens on artificial media. However, it is often difficult to identify the underlying pathogen based on those characters alone. For example, the disease symptoms of *Verticillium* wilt in hemp is very similar to *Fusarium* wilt and the pathogen species in both genera can invade a wide range of economical crops [51][52][53]. In addition, it is difficult to distinguish the species within most fungal genera based on morphological features alone. However, most of them are relatively easy to identify using molecular markers, as described below (**Table 2**; **Table 3**).

Table 3. Genes and PCR primers used for their amplification in fungal pathogens infecting bast fiber crops.

Target DNA	Primer Name and Sequence (5′-3′)		Size of PCR Product (bp)	Reference
18S	NS3	GCAAGTCTGGTGCCAGCAGCC	Not mentioned	[54]
	NS4	CTTCCGTCAATTCCTTTAAG		
28S	LR0R	GCAAGTCTGGTGCCAGCAGCC	Not mentioned	[54]
	LR3	GCAAGTCTGGTGCCAGCAGCC		
25S	LROR	ACCCGCTGAACTTAAGC	1431	[33]
	LR7	TACTACCACCAAGATCT		
ACT	ACT-512F	ATGTGCAAGGCCGGTTTCGC	300	[25]
	ACT-783R	TACGAGTCCTTCTGGCCCAT		
ß-tubulin	Vd-btub-1F	GCGACCTTAACCACCTCGTT	Not mentioned	[52]
	Vd-btub-1R	CGCGGCTGGTCAGAGGA		
	VertBt-F	AACAACAGTCCGATGGATAATTC	Not mentioned	[52]
	VertBt-R	GTACCGGGCTCGAGATCG		
	VITubF2	GCAAAACCCTACCGGGTTATG	143	[53]
	VITubRI	AGATATCCATCGGACTGTTCGTA		

Target DNA	Primer Name and Sequence (5′-3′)		Size of PCR Product (bp)	Reference
CAL	VdTubF2	GGCCAGTGCGTAAGTTATTCT	82	[53]
	VdTubR4	ATCTGGTTACCCTGTTCATCC		
	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	Not mentioned	[27]
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC		
	CL1	GARTWCAAGGAGGCCTTCTC	Not mentioned	[27]
	CL2	TTTTTGCATCATGAGTTGGAC		
	CAL-228F	GAGTTCAAGGAGGCCTTCTCCC	Not mentioned	[45]
	CAL-737R	CATCTTTCTGGCCATCATGG		
EF-1α	EF-1	ATGGGTAAGGAGGACAAGAC	700	[37]
	EF-2	GGAGGTACCAGTGATCATGTT		
	EF1-728F	CATCGAGAAGTTCGAGAAGG	Not mentioned	[45]
	EF2	GGAGGTACCAGTGATCATGTT		
	EF1-728F	CATCGAGAAGTTCGAGAAGG	350	[25]
	EF1-983R	TACTTGAAGGAACCCTTACC		

Target DNA	Primer Name and Sequence (5′-3′)		Size of PCR Product (bp)	Reference
Endochitinase	Vd-endoch-1F	CTCGGAGGTGCCATGTACTG	Not mentioned	[52]
	Vd-endoch-1R	ACTGCCTGGCCCAGGTTC		
GAPDH	Vd-G3PD-2F	CACGGCGTCTTCAAGGGT	Not mentioned	[52]
	Vd-G3PD-1R	CAGTGGACTCGACGACGTAC		
	GDF1	GCCGTCAACGACCCCTTCATTGA	Not mentioned	[27]
	GDR1	GGGTGGAGTCGTACTTGAGCATGT		
	gpd-1	CAACGGCTTCGGTCGCATTG	Not mentioned	[24]
	gpd-2	GCCAAGCAGTTGGTTGTGC		
GS	GSF1	ATGGCCGAGTACATCTGG	Not mentioned	[27]
	GSR1	GAACCGTCGAAGTTCCAC		
ITS	ITS1	TCCGTAGGTGAACCTGCGG	334-738	[30][28][48][49][51][52]
	ITS4	TCCTCCGCTTATTGATATGC		
	Vd-ITS1-45-F	CCGGTCCATCAGTCTCTCTG	334	[51]

Target DNA	Primer Name and Sequence (5′-3′)		Size of PCR Product (bp)	Reference
	Vd-ITS2-379-R	ACTCCGATGCGAGCTGTAAC		
	ITS1-F	CTTGGTCATTTAGAGGAAGTAA	700	[37]
	ITS4 [33]	TCCTCCGCTTATTGATATGC		
	VtF4	CCGGTGTTGGGGATCTACT	123	[53]
	VtR2	GTAGGGGGTTTAGAGGCTG		
	ITS 4	TCCTCCGCTTATTGATATGC	Not mentioned	[27]
	ITS 5	GGAAGTAAAAGTCGTAACAAGG		
RPB2	bRPB2-6F	TGGGGYATGGTNTGYCCYGC	Not mentioned	[34]
	bRPB2-7R	GAYTGRTTRTGRTCRGGGAAVGG		

pecifically, digestion *cannabis* ogens on phasis on were low bast fiber crops [55]. from bast

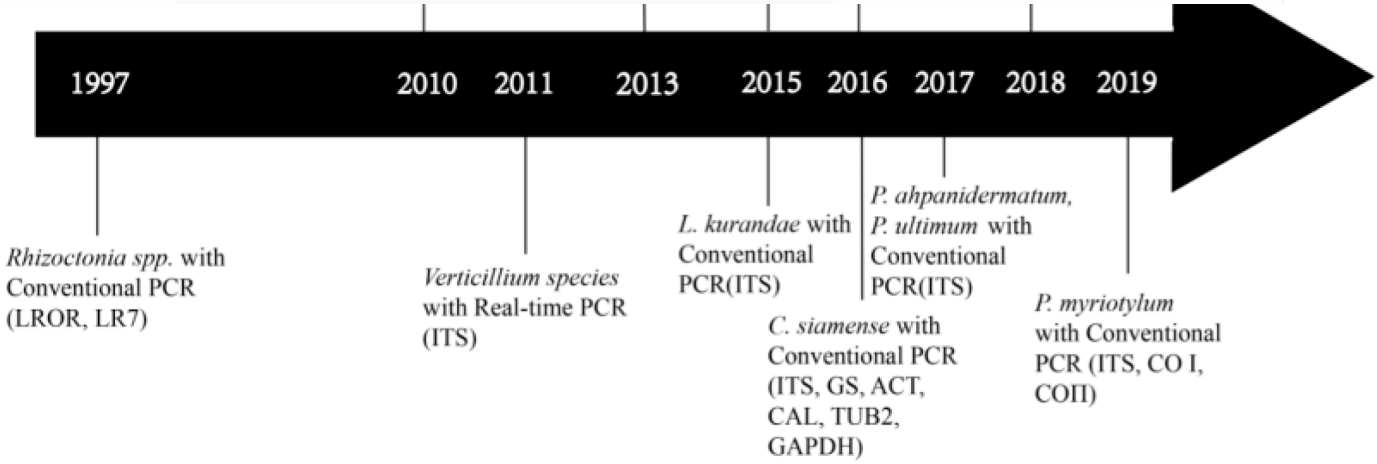

Figure 1. Development of molecular-based assays for the detection of fungal pathogens in bast fiber crops from 1997 until the present. For genus and species names, please see text and **Table 2**. Details of primers are shown in **Table 3**.

According to the National Center for Biotechnology Information (NCBI) PubMed, the most common literature on the molecular identification of fungal pathogens on bast fiber crops has been on hemp (including both industrial hemp and medicinal marijuana), accounting for ~45% of all published articles. This was then followed by flax and kenaf (at ~14% each), ramie (11%), and the rest being jute and sunn hemp. However, most of these reports were case reports.

5. Target DNA Selection and Molecular Assays of Fungal Pathogens on Bast Fiber Crops

Over the last three decades, several types of DNA-based methods have been developed and widely used to detect plant fungal pathogens. The invention of PCR technology using a thermostable polymerase by Kary Mullis gave birth to PCR in the early 1980s [4]. The invention of PCR has led to a diversity of PCR-based methods for fungal pathogen detections based on variations in DNA sequences within and among species (**Figure 1**, **Table 2**). Among these methods, qPCR is probably the most common molecular technology and it can be used for quantitative measurement of RNA and DNA, targeting both single nucleotide polymorphisms (SNPs) and copy number variations. qPCR allows not only the detection of whether a specific pathogen(s) is present in the sample, but also the quantification of pathogen levels in host tissues [5][6]. To improve the efficiency of conventional PCR, other methods have been coupled with PCR for plant fungal pathogen detection. For example, PCR in combination with enzyme-linked immunosorbent assay (ELISA) has been successfully applied to detect fungi, viruses, and bacteria, with high specificity [56]. Similarly, the highly specific IC-PCR approach can increase the sensitivity by 250 folds compared to conventional PCR amplification [7][8]. For absolute quantification without the need for references and standard curves, dd-PCR is the method of choice—this method is based on the combined technology of water–oil emulsion droplet and PCR [9]. In field conditions without ready access to laboratory equipment, LAMP can provide fast identifications of samples. LAMP uses six primers that are highly specific to target sites in a specific gene [10]. It can be carried out at a constant temperature in a short reaction time (<30 min). It is sensitive and cost-effective, potentially making it an ideal method for field detection of plant pathogens [57].

As shown in **Table 2**, PCR-based methods have been used as the main approach for detecting fungal pathogens in bast fiber crops. This pattern is similar to the detections of fungal pathogens in other crops in general. A number of DNA fragments and genes have been explored as potential targets for PCR-based detections, including the ribosomal RNA gene cluster, conserved housekeeping genes, and genes involved in the production of secondary metabolites, including mycotoxins [58][59][60]. **Table 3** summarizes the genes and their primers that have been used for the detection and diagnostics of fungal pathogens on bast fiber crops. The researchers would like to note that the molecular analyses reported so far for identifying fungal pathogens on bast fiber crops have been primarily using pure fungal strains, not those from diseased plant tissues. There is a large gap in applying these molecular methods in field conditions as a point-of-care test.

Among the DNA fragments that have been used for fungal pathogen detection, the most frequently used is the ribosomal RNA gene cluster. This gene cluster is composed of up to hundreds of repeating units with each unit containing the genes encoding the small (18S) ribosomal RNA subunit, the internal transcribed spacer (ITS)

regions 1 and 2 that are separated by the 5.8S rRNA subunit, and the large (28S) ribosomal RNA subunit, with the intergenic spacer (IGS) region separating the adjacent units (**Figure 2**). The entire ITS fragment (which comprises ITS1, 5.8S rRNA, and ITS2) is typically 500–750 bp long and flanked by the 18S and 28S rRNA genes [61][62][63]. The ITS regions are present in all known fungi and have both highly conserved flanking regions located in the 5.8S, 18S, and 28S rRNA genes as well as the variable regions (located in the ITS1 and ITS2 regions). The conserved flanking regions allowed the development of highly conserved probes or primers to amplify most, if not all, fungi, while the variable regions allowed the development of species-specific markers [64][65]. Together, these features have contributed to ITS being the consensus fungal DNA barcode for the mycological community [64][65]. Furthermore, the ITS sequences obtained from the direct amplification and sequencing of environmental DNA samples have contributed to our increased understanding of fungal diversity from a variety of environments, including those from diseased plants and animals [65][66].

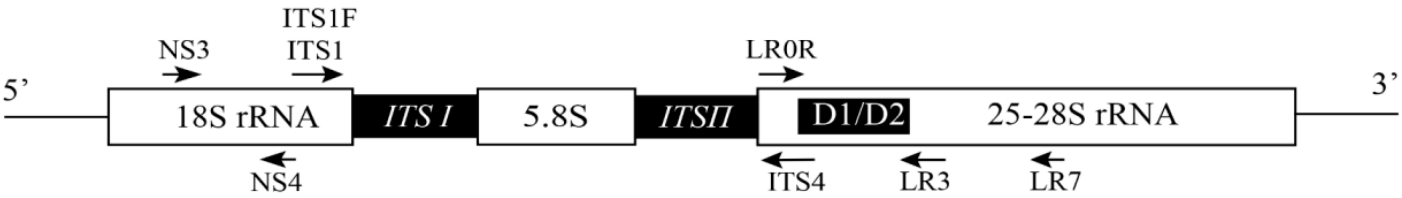

Figure 2. A schematic representation of the fungal ribosomal RNA gene cluster showing the locations of individual DNA fragments and the common primers used for PCR amplification.