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Characterization of blast resistance related protein domains in wheat for molecular marker development

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Abstract

Wheat blast is a devastating disease which is baffling scientists from its inception. This study characterized the blast resistance related protein domains with a view to develop molecular markers to identify resistant wheat genotypes against Blast fungus *Magnaporthe oryzae*. A genome browse analysis detected that the candidate resistance gene against blast could be located in several different chromosomes. An *in silico* analysis was collected with fifty nucleotide-binding site leucine-rich repeat (NBS-LRR), leucine-rich repeat (LRR), pathogenesis and resistance protein-encoding accessions on the basis of the previous resistance report. The phylogenetic tree of those putative resistance accessions, bearing resistance related protein-encoding domains, showed that an NBS-LRR accession JP957107.1 has 67% similarity with the disease resistance protein domain encoding accession of Brazilian resistant cultivar *Thatcher*. By contrast, the rice blast resistance *Pita* gene has 72% similarity with 18 pathogenesis protein domain encoding accessions. Among putative protein domains, disease resistance protein of *Thatcher* has 78% similarity with two NBS-LRR protein domains AAZ99757.1 and AAZ99757.1. Eighteen microsatellite markers were designed from eighteen putative NBS-LRR protein encoding accessions along with *Piz3* marker. The 19 markers were unable to separate resistant and susceptible genotypes. Diffused versus conspicuous bands indicated either presence of insertion/deletion (InDel) or single nucleotide polymorphism (SNP) among wheat genotypes. Detection of InDel or SNP markers is a subject of further investigation. Additional markers are needed to be designed using new NBS-LRR, pathogenesis, coiled-coil (CC), translocated intimin receptor (TIR) resistance protein encoding accessions to find out markers specific for blast resistance.

Introduction

Blast is a serious disease of major cereal producing poaceae family members including wheat and rice. In Latin, blast is called as "Brusone" (Couch and Kohn, 2002). *Magnaporthe oryzae* pathotype *Triticum* fungus is accountable for blast disease in wheat (Malaker *et al.*, 2016). The first occurrence of wheat blast was noticed in 1985 and Parana province of Brazil was the hotspot (Igarashi, 1986). In February 2016, Bangladesh became the first wheat blast affected South-Asian country (Callaway, 2016). For wheat blast disease crop production can be dwindled from 40% to 100% (Kohli *et al.*, 2011; Goulart and Paiva, 1992). After first blast outbreak in 1985 in Brazil, scientists started searching for sources of resistance to resist this terrifying disease but the way was much harder (Urashima *et al.*, 2004; Prestes *et al.*, 2007; Cruz *et al.*, 2010, 2016). The resistance against blast pathogen depends on R gene. The main problem based on R gene resistance is "breakdown of resistance" due to the high level of pathogenic variation within blast pathogen (Kiyosawa, 1982; Leach *et al.*, 2001). Global warming is one of the significant reasons for dissemination and development of blast disease (Kohli *et al.*, 2011).

Most of the disease resistance genes in plants encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins (Mchale *et al.*, 2006, Laila *et al.*, 2019). Hypersensitive reaction to a pathogen triggered by pathogenesis related protein which is one of the most efficient defense mechanism (Stintzi *et al.*, 1993). In case of rice blast, it has found that NBS-LRR protein domain has role in resistance and specifically nineteen blast resistant genes encode NBS-LRR protein domain (Jiang *et al.*, 2012). So it's urgent to search and characterize protein domains related with blast resistance. The study was conducted to explore the structure and interaction pattern of resistance related domains including NBS-LRR, leucine-rich repeat (LRR), coiled-coil (CC), translocated intimin receptor (TIR), pathogenesis, and resistance protein domain for developing microsatellite markers to identify genotypes resistant to wheat blast.

Materials and Methods

In silico analysis

Desired list of protein-encoding domains were collected from NCBI, Ensembl Plants and Gramene databases

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along with their accession numbers (Ware *et al.,* 2002*;* Pruitt *et al.,* 2006; Bolser *et al.,* 2016; Table 1). For illustration of the accessions with exon-intron and flanking regions the GSDS online software was used (Guo *et al.,* 2007). The BLAST similarity search was conducted to find out syntenic locations of disease resistance protein-encoding accession of *Thatcher* genotype (GenBank accession: KY064065.1) and *Pita* gene (GenBank accession: AY196754.1) (Bolser *et al.,* 2016). *Thatcher* is a blast resistant Brazilian wheat genotype containing two R genes *Rmg2* and *Rmg3* (Zhan *et al.,* 2007). After that, *Pita* is a rice blast resistant gene. *Pita* is a monogenic qualitative R gene which is also known as *Rmg16* found in *Japonica* rice. Previously from *Pita* gene, *Piz3* marker was developed and utilized against rice blast (Imam *et al.,* 2014). Finally, phylogenetic analysis was performed in online software Phylogeny.fr (Dereeper *et al*., 2008). "HSP distribution on genome" was obtained for disease resistance protein-encoding accession of *Thatcher* and *Pita* through BLAST search.

Table 1. List of retrieved accessions from NCBI with the protein encoding domains

Serial no.	Accession no.	Protein type	Serial no.	Accession no.	Protein type
1	KY064065.1	Disease resistance protein-encoding	26	GUOS4176.1	LRR protein encoding
2	AY196754.1	Rice blast resistant	27	DQ097959.1	LRR protein encoding
3	KX181569.1	NBS-LRR protein encoding	28	AJ606035.1	LRR protein encoding
4	DQ128005	NBS-LRR protein encoding	29	HQ541963.1	Pathogenesis protein encoding
5	AF320847.1	NBS-LRR protein encoding	30	AY258615.1	LRR protein encoding
6	DQ145755	NBS-LRR protein encoding	31	KX673535.1	Pathogenesis protein encoding
7	KF810140.1	NBS-LRR protein encoding	32	KX673549.1	Pathogenesis protein encoding
8	DQ128021.1	NBS-LRRprotein encoding	33	KX673548.1	Pathogenesis protein encoding
9	DQ128020.1	NBS-LRR protein encoding	34	KX673547.1	Pathogenesis protein encoding
10	DQ128017.1	NBS-LRR protein encoding	35	KX673546.1	Pathogenesis protein encoding
11	DQ128002.1	NBS-LRR protein encoding	36	KX673543.1	Pathogenesis protein encoding
12	DQ128000.1	NBS-LRR protein encoding	37	KX673539.1	Pathogenesis protein encoding
13	DQ127990.1	NBS-LRR protein encoding	38	KX673538.1	Pathogenesis protein encoding
14	DQ120792.1	NBS-LRR protein encoding	39	KX673537.1	Pathogenesis protein encoding
15	AY555270.1	NBS-LRR protein encoding	40	KX673536.1	Pathogenesis protein encoding
16	AY238935.1	NBS-LRR protein encoding	41	KF196311.1	Pathogenesis protein encoding
17	AY212115	NBS-LRR protein encoding	42	HQ700377.1	Pathogenesis protein encoding
18	AF525278	NBS-LRR protein encoding	43	HQ541981.1	Pathogenesis protein encoding
19	JF957107	NBS-LRR protein encoding	44	HQ541979.1	Pathogenesis protein encoding
20	GU356591	NBS-LRR protein encoding	45	HQ541976.1	Pathogenesis protein encoding
21	KY784576.1	LRR protein encoding	46	HQ541965.1	Pathogenesis protein encoding
22	KX840357.1	LRR protein encoding	47	AY270159.1	Pathogenesis protein encoding
23	KC700616.1	LRR protein encoding	48	AY270158.1	Resistance protein encoding
24	KC70015.1	LRR protein encoding	49	AY270157.1	Resistance protein encoding
25	JN872563.1	LRR protein encoding	50	DQ205351.2	Resistance protein encoding

Marker design

Eighteen NBS-LRR protein domain encoding accessions were selected from NCBI database to design primers to develop PCR-based markers. Primers were designed using online tool Primer3plus (Table 2). The Primer3plus online tool picked up forward and reverse primers with the product size between 150 and 250 bp and annealing temperature between 58^{o}C and 61^{o}C (Table 2, Untergasser *et al.,* 2007). *Piz3* primer referring *Pita* gene was collected from Gramene database (Imam *et al.,* 2014). The relevant information of 18 designed markers and *Piz3* microsatellite marker are given in Table 2.

Marker Validation

Collection of genotypes and extraction of DNA

A total of 18 wheat genotypes belongs to *Triticum aestivum* were used in this experiment (Table 3). Among those varieties BARI Gom 33 has blast resistance, Bari Gom 31-32 have moderate blast tolerance and Bari Gom 25-30 have susceptibility to blast (DGGW, 2017, Personal Communication, NCD Barma, Director, Bangladesh Wheat and Maize Research Institute). The modified Cetyl Trimethyl Ammonium Bromide (CTAB) mini-prep method was used to extract genomic DNA from the wheat leaves (Zhang *et al.,* 2013).

Table 2. Details of the designed microsatellite markers (SSRs) for validation

Primer Name	GenBank accession		Sequence (5´- 3´)	Annealing Temp. (°C)	Expected PCR Product Size
M1	AF525278.1	Fwd.	TGTCACAATCATGCGACCTT	58	167
		Rev.	GCTTGGCCAACTCATTAACC		
M2	DQ145755.1	Fwd.	AAGGCTCAACTTACCGTGGA	58	170
		Rev.	TGTTGTGAGGATCAGCTTGC		
M3	DQ128021.1	Fwd.	GCGGGGAGAGAGATACAAGA	58	156
		Rev.	AGCGAGTCCGACACCTAGAG		
M4	DQ128020.1	Fwd.	TTGCAGGAGATGTTGTGCTC	58	233
		Rev.	GCAACCCTTTTGCTTTGTGT		
M5	DQ128019.1	Fwd.	GCGCTCTTVTGAAAGGAAATG	58	222
		Rev.	GCAACCCTTTTGCTTTGTGT		
M6	DQ128017.1	Fwd.	AAGCGGTTGTTGCTTGTTTT	58	193
		Rev.	CCCCATGACTTAGACGTTCC		
M7	DQ128007.1	Fwd.	GCAAGCTGATCCTCACAACA	58	153
		Rev.	GCCATCCAACTGATGCACTA		
M8	DQ128005	Fwd.	GCAAGCTGATCCTCACAACA	58	171
		Rev.	TACCATTCTGGGAACCAAGC		
M9	DQ128002.1	Fwd.	CTTTCACCCAACGCCTGTAT	58	189
		Rev.	GCGCAATGGATTTTTGAAGT		
M10	DQ128000.1	Fwd.	TGTGCTTGATGATGTGTGGA	58	157
		Rev.	GCTACCCAATGGCTGGAGTA		
M11	DQ127998.1	Fwd.	GGTACATATCGCCGTGCTCT	58	190
		Rev.	TTGGGTGTGCATTGGAGTTA		
M12	DQ127990.1	Fwd.	AGAGAAGGACAGGGGTGGAT	58	177
		Rev.	GCCTTGAAAAACAGCCAAAA		
M13	KF810141.1	Fwd.	CTGCGTACAAAGACCAGCAA	58	162
		Rev.	ATCCTTGGGAAACACAGCAC		
M14	KX181569.1	Fwd.	ACTCATCCGGTCGTTACCAG	58	150
		Rev.	GACACCAGGGTAGGGCAGTA		
M15	DQ120792.1	Fwd.	AAGCATTTCCAGTTGCTGCT	60	170
		Rev.	TCCAGGACAAGGAGATACCG		
M16	JF957107.1	Fwd.	CAGGTGGGAAAGAAGATCCA	58	236
		Rev.	TCCCAACTCCTCGTTTCATC		
M17	KF810140.1	Fwd.	GAGTGCAGCAATGTTGGAGA	58	172
		Fwd.	CCCTTGCATCATCCCTAGAA		
M18	AY238935.1	Fwd.	ATCGCTGACCATTTTGAAGC	58	220
		Rev.	CCTGTAGCTGCTCCCTTTTG		
*Piz*3	AY196754.1	Fwd.	AGTCGTGCGATGCGAGGACAGAAAC	62	861
		Rev.	GCATTCTCCAACCCTTTTGCATGCAT		

Table 3. List of wheat genotypes collected from Bangladesh Agriculture Research Institute (BARI) used to assess resistant and susceptible wheat genotypes against wheat blast disease

Sl. No.	Name of the wheat genotypes	Type
1	BARI Gom 21	High yielding variety (HYV)
2	BARI Gom 22	HYV
3	BARI Gom 23	HYV
4	BARI Gom 24	HYV
5	BARI Gom 25	HYV
6	BARI Gom 26	HYV
7	BARI Gom 27	HYV
8	BARI Gom 28	HYV
9	BARI Gom 29	HYV
10	BARI Gom 30	HYV
11	BARI Gom 31	HYV
12	BARI Gom 32	HYV
13	BARI Gom 33	HYV
14	Sonalika	HYV
15	Gourav	HYV
16	Sourav	HYV
17	Aghrani	HYV
18	Kheri	Landrace

PCR amplification and agarose gel electrophoresis

Addbio® Taq Master Mix was used to amplify genomic DNA of 18 wheat genotypes. Each 10 µl PCR reaction included 1 µl DNA, 2 µl of each primer, 3.5 µl PCR master mix and 3.5 µl sterile distilled water. Reactions were pre-incubated for 5 min at 94°C followed by 35 cycles of amplification at 95°C for 45 s, 58-62 °C for 45 s and 72°C for 45 s in the GeneAtlas thermal cycler (Astec Co. Ltd, South Korea). Electrophoresis of PCR product was done by using 1.5% agarose.

Results and Discussion

Putative disease resistance protein encoding accession of *Thatcher* cultivar and its synteny in wheat genome

The sequence of putative disease resistance protein encoding gene of *Thatcher* accession is 2739 bp (Pruitt *et al.*, 2006). The disease resistance protein-encoding accession of *Thatcher* showed best match between 24401845 and 24404427 bp with 99.8% identity on unknown chromosome of wheat (Bolser *et al.*, 2016).

The paralogs of that resistance protein domain-encoding accession of *Thatcher* were distributed throughout the wheat genome except 1D, 3A, 4D, 6D and 7D chromosomes indicating that resistance related to wheat blast might be located in any of those chromosomes. But this speculation is a subject of further investigation (Fig. 1).

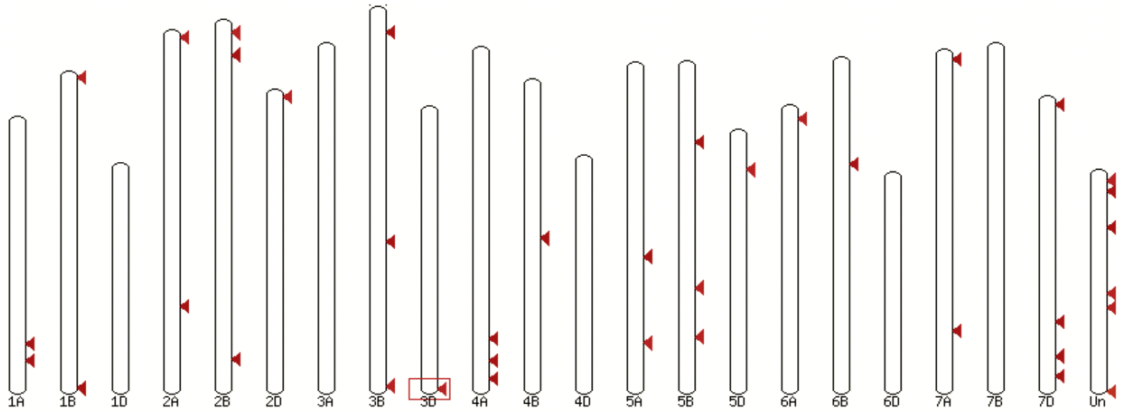

Fig. 1. Distribution of the paralogs of disease resistance accession of *Thatcher* in the whole genome of wheat. BLAST search was conducted in wheat genome of Ensembl Plants where disease resistance accession of *Thatcher* was used as reference sequence. Highest number of hits was found in the unknown chromosome of wheat. The paralog of disease resistance accession of *Thatcher* was completely absent in 1D, 3A, 4D, 6D and 7D chromosomes of wheat.

Putative rice blast resistance gene *Pita* and its synteny in wheat genome

The total sequence size of the *Pita* gene was 5113 bp (Ware *et al.*, 2002). The *Pita* gene showed the best match in the 5A chromosome between 548608286 and 548608336 bp of wheat genome with 90.2% identity in 5A chromosome of wheat (Bolser *et al.*, 2016). The orthologs of *Pita* gene were distributed in all chromosomes of wheat genome except for 1A chromosome. The highest frequency of the paralogs was seven and was found in 1D, 4A, 7A, 7B and 7D chromosomes of the wheat genome (Fig. 2).

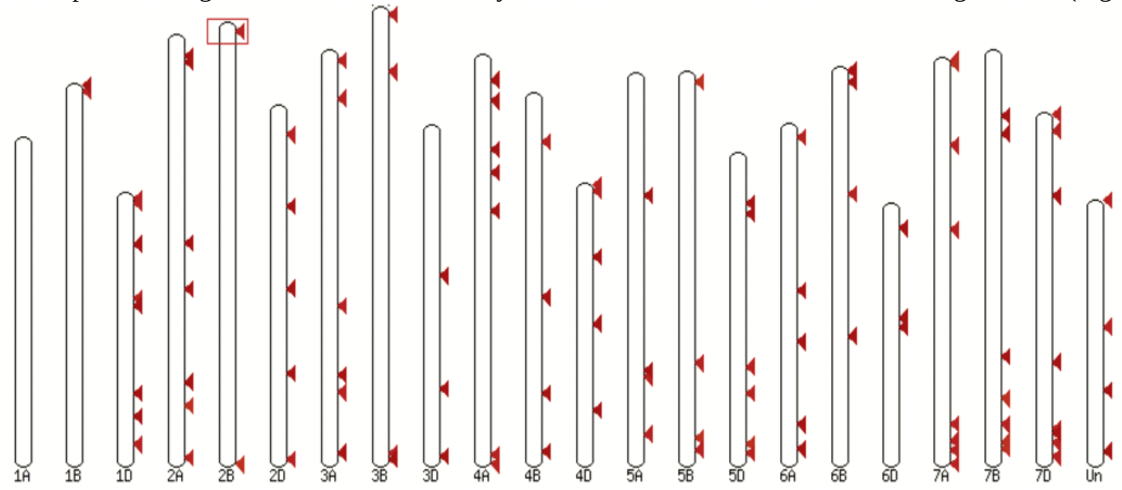

Fig. 2. Distribution of the orthologs of *Pita* gene in the whole genome of wheat. BLAST search was conducted in wheat genome of Ensembl Plants where *Pita* gene sequence was used as reference sequence. Highest hits were found in 1D, 4A, 7A, 7B and 7D chromosomes of the wheat genome and totally absent in 1A chromosome.

Exon-intron distribution of resistance-protein encoding sequences

The smallest NBS-LRR protein coding accession among the fifty selected accessions was DQ128021.1 with 194 bp size and KX181569.1 was the largest NBS-LRR protein-encoding accession with 3390 bp size. The intron was present in three NBS-LRR protein encoding accession (DQ128005, DQ145755, and KF810140.1) (Fig. 3).

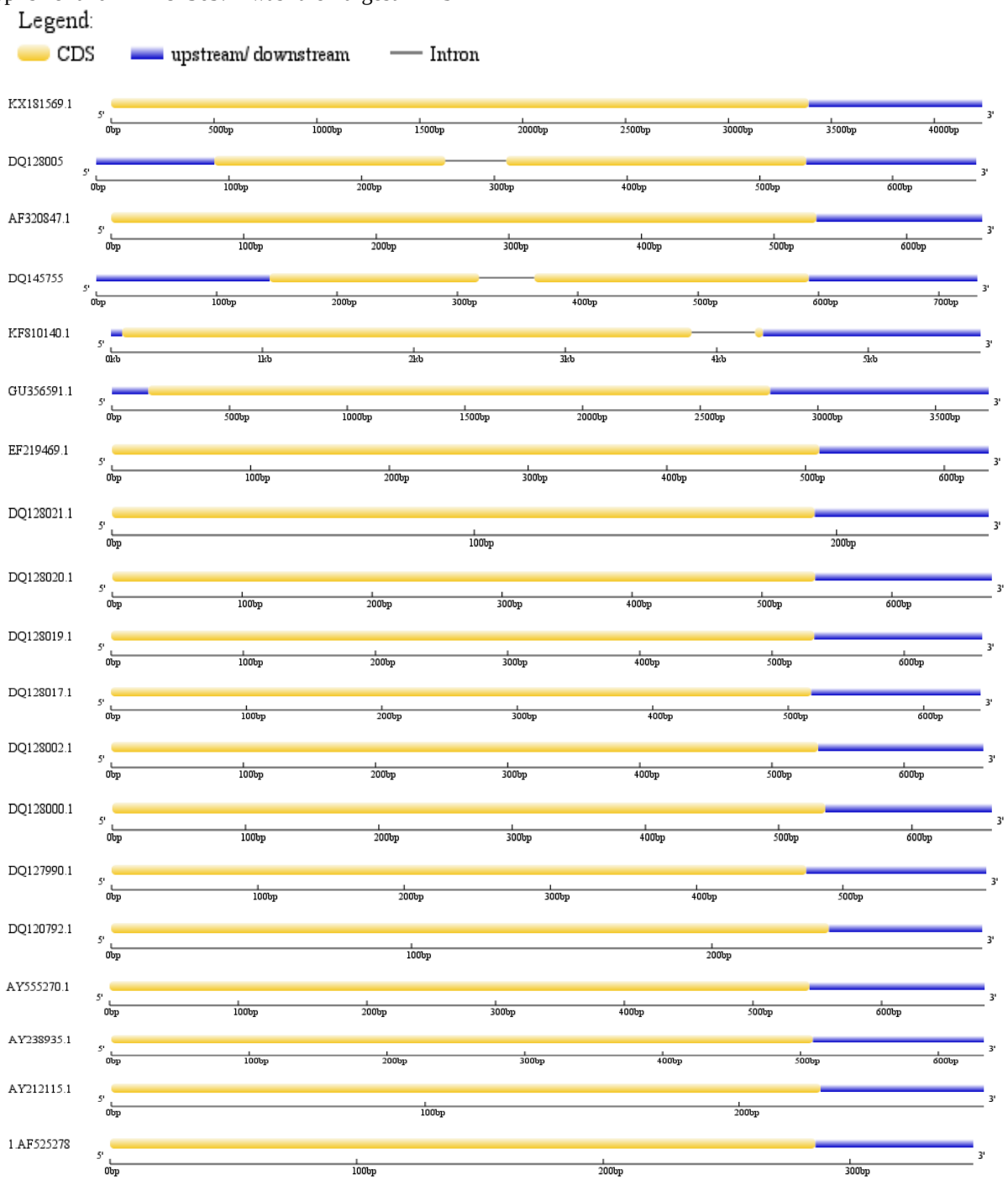

Fig. 3. Exon-intron distribution of accessions encoding NBS-LRR protein domain. GenBank accessions: KX181569.1, DQ128005, AF320847.1, DQ145755, KF810140.1, DQ128021.1, DQ128020.1, DQ128017.1, DQ128002.1, DQ128000.1, DQ127990.1, DQ120792.1, AY555270.1, AY238935.1, AY212115, AF525278

Among eight LRR protein encoding accessions, DQ097959.1 was the smallest and KY784576.1 was the largest. The intronic sequence was absent in eight accessions (Fig. 4).

Among eighteen pathogenesis protein-encoding accessions, AY258615.1 was the smallest with the size of 560 bp and KX673535.1 was the largest pathogenesis protein-encoding accession with the size of 2241 bp (Fig. 5).

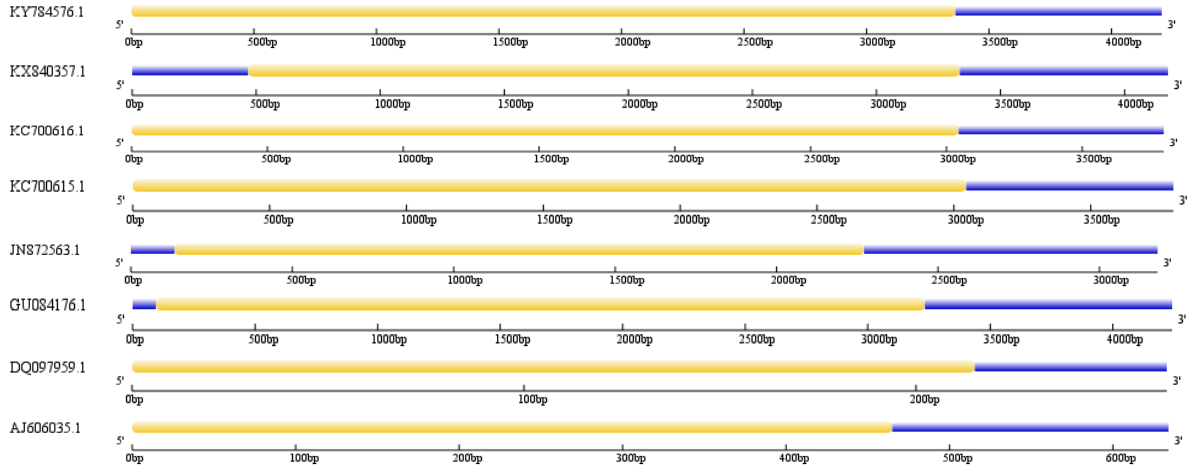

Fig. 4. Exon-intron distribution of protein-encoding LRR accessions. GenBank accessions: KY784576.1, KX840357.1, KC700616.1, KC70015.1, JN872563.1, GUOS4176.1, DQ097959.1, AJ606035.1

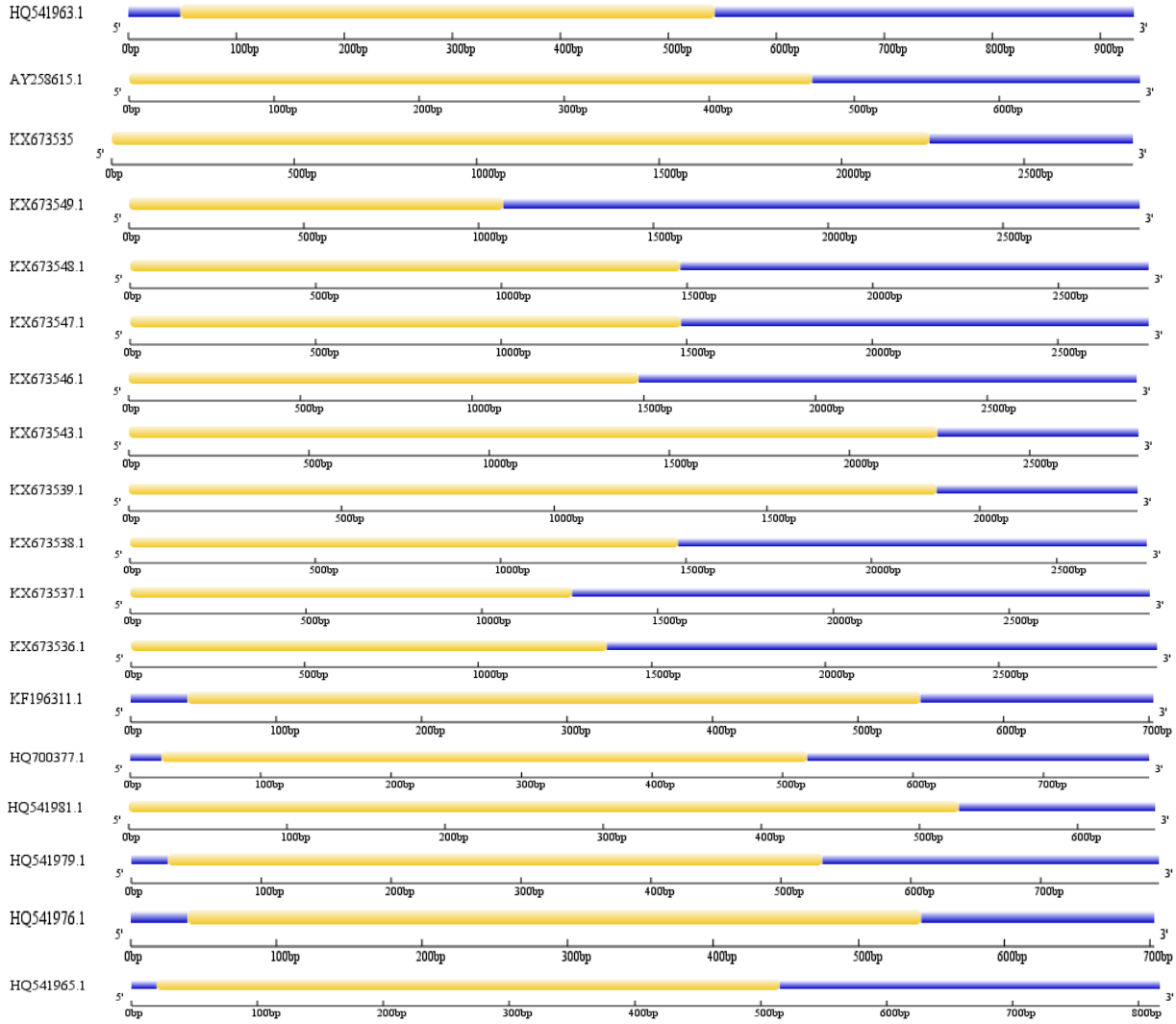

Fig. 5. Exon-intron distribution of accessions encoding pathogenesis protein domain. GenBank accessions: HQ541963.1, AY258615.1, KX673535.1, KX673549.1, KX673548.1, KX673547.1, KX673546.1, KX673543.1, KX673539.1, KX673538.1, KX673537.1, KX673536.1, KF196311.1, HQ700377.1, HQ541981.1, HQ541979.1, HQ541976, HQ541965.1

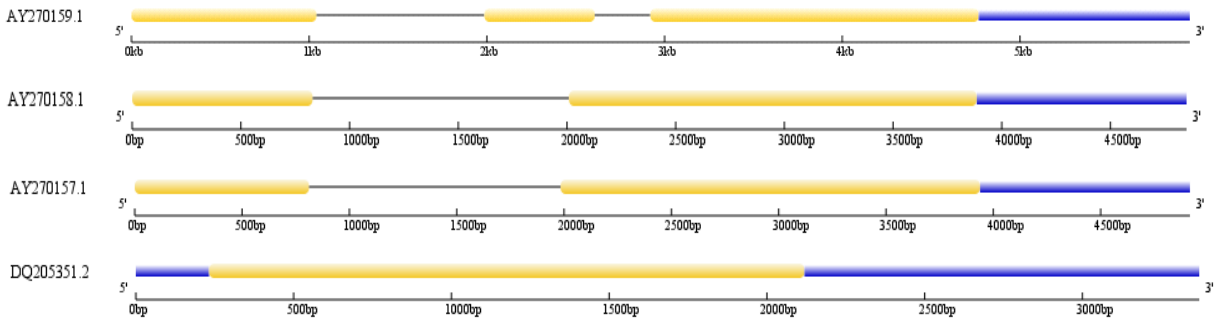

Fig. 6. Exon-intron distribution of resistance protein-encoding accessions. GenBank accessions: AY270159.1, AY270158.1, AY270157.1, DQ205351.2

AY270159.1, AY270158.1, AY270157.1, DQ205351.2 were four disease resistance protein-encoding accessions. Among these four accessions, DQ205351.2 was the smallest (2693 bp) and AY270159.1 was the largest (4768 base pair) (Fig. 6).

The retrieved NBS-LRR, pathogenesis, LRR, resistance protein-encoding accessions could be a potential source of blast resistance. The majority of NBS-LRR protein domains encode resistance genes. The sub-classes of NBS-LRR, CC and TIR have a role in disease resistance (Mchale *et al.*, 2006; Marone *et al.*, 2013). Furthermore, the pathogenesis protein encoding genes have a significant role against stress response by hypersensitive reaction (Van Loon, 1985; Stintzi *et al.*, 1993). So, it can be expected that these retrieved putative protein encoding accessions might be useful for blast resistance in wheat. Further study is needed to identify the role of those protein domain encoding accessions.

Phylogenetic relationship between resistances related protein coding accessions

The phylogenetic relatedness showed resistance related protein encoding domain of *Thatcher* (KY064065.1) is related with JP957107.1 with a 0.67 bootstrap value which indicated both accessions had 67% similarity (Fig. 7). Eighteen putative pathogenesis related protein encoding accessions HQ700377.1, KF196311.1, HQ541979.1, HQ541976.1, HQ541965.1, HQ541963.1, AY258615.1, HQ541981.1, KX673535.1, KX673536.1, KX673537.1, KX673538.1, KX673539.1, KX673543.1, KX673546.1, KX673547.1, KX673548.1, KX673549.1), two putative NBS-LRR protein encoding accessions (KF810140.1, KF810141.1), and two putative LRR protein encoding accessions (KX840357.1, AJ606034.1) were related with rice blast resistant gene *Pita* (AY196754.1) with 0.72 bootstrap value that showed 72% similarity (Fig. 7). Phylogenetic analysis was broadly used to find out the closeness of genes and protein domains (Cavalli‐sforza and Edwards, 1967; Pan *et al.*, 2000). It is expected that the identified protein encoding accessions of *Thatcher* and *Pita* might carry resistance for blast disease.

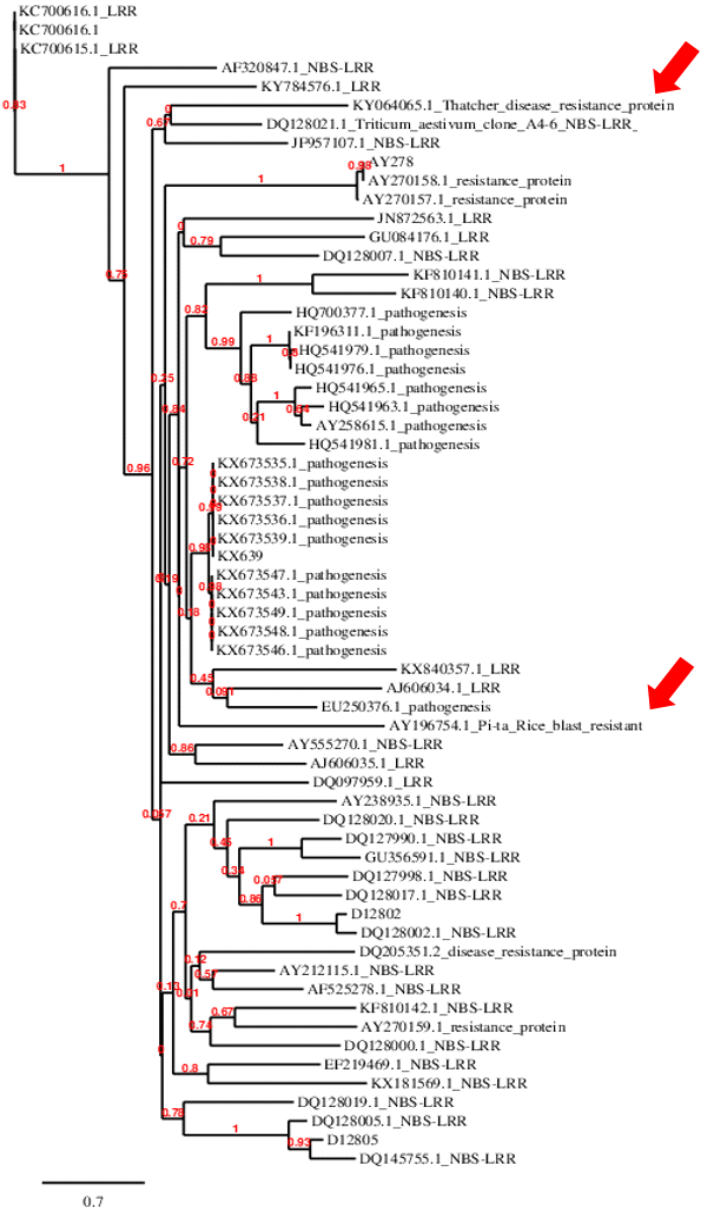

Fig. 7. Phylogenetic tree was constructed using Phylogeny.fr online software. Retrieved putative protein-encoding accessions of wheat genome was utilized for constructing phylogenetic tree where disease resistance protein encoding accession of *Thatcher* and *Pita* gene had taken as reference sequence. A NBS-LRR protein encoding accession (JP957107.1) had 67% similarity with disease resistance protein encoding accession of *Thatcher*. Likewise, *Pita* gene had 72% similarity with 22 protein encoding accessions including 18 pathogenesis, two NBS-LRR and two LRR protein domain-encoding genes.

Phylogenetic relationship among protein domains

The putative disease resistant protein of *Thatcher* (AA045168.1) was closely related with two NBS-LRR protein domain (AAZ99757.1, AAZ997575.1) with a 0.78 bootstrap value that showed 78% similarity (Fig. 8). Disease resistance protein of *Thatcher* genotype (AR038245.1), two other putative disease resistance proteins (AAQ01784.1, AAQ01785.1) and two putative NBS-LRR protein domains (AAZ9975.1, AAZ99757.1) were related with *Pita* protein with 0.43 bootstrap value indicating 43% similarity (Fig. 8).

So, the closely related proteins of *Pita* and disease resistance protein of *Thatcher* might bear resistance for blast. Further study is needed to validate the findings.

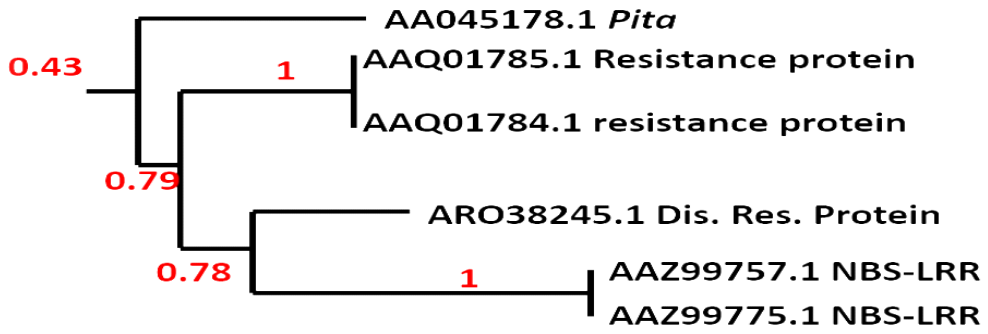

Fig. 8. Phylogenetic tree was constructed using Phylogeny.fr online software. Retrieved putative protein accessions of wheat genome was utilized for constructing phylogenetic tree where disease resistance protein of *Thatcher* and *Pita* protein had taken as reference sequence. *Pita* protein had 43% similarity with five protein domains including disease resistance protein of *Thatcher*. Disease resistance protein of *Thatcher* had 78% similarity with two NBS-LRR protein domains.

Marker validation

Eleven markers M2, M3, M4, M5, M6, M7, M11, M13, M14, M16, and M18 respectively amplified genomic DNA of all eighteen wheat genotypes. There was no variation among eighteen genotypes except dark and light DNA bands. The PIC value of all those markers was 0.00. The M1 marker was run for eighteen wheat genotypes and was found to amplify genomic DNA of 14 genotypes except BARI Gom 24, BARI Gom 29, BARI Gom 31 and BARI Gom 32 (Fig. 9). The M8 marker amplified ten genotypes except BARI Gom 31, BARI Gom 32, BARI Gom 33, Sonalika, Protiva, Sourav, Kheri, Kanchan (Fig. 9). The M9 marker amplified genomic DNA of sixteen genotypes except BARI Gom 29 and BARI Gom 30 (Fig. 9). The M10 marker amplified genomic DNA of all genotypes except BARI Gom 29 and BARI Gom 30 (Fig. 9). The M15 marker amplified genomic DNA of all genotypes except BARI Gom 29. M17 marker amplified genomic DNA of all wheat genotypes except BARI Gom 23, BARI Gom 28, BARI Gom 29, BARI Gom 30 and BARI Gom 31 (Fig. 9). *Piz3* gene marker amplified genomic DNA of ten genotypes except BARI Gom 21, BARI Gom 23, BARI Gom 27, BARI Gom 28, BARI Gom 29, Sonalika, Protiva, Sourav and Kheri (Fig. 9). M8 and *Piz3* markers estimated the highest PIC value of 0.49. The PIC values of M1, M9, M10, M12, M15, and M17 markers were 0.35, 0.20, 0.20, 0.24, and 0.40 respectively.

Previously, NBS-LRR protein domain utilized for the development of rice blast resistant marker and it could be noted that around nineteen NBS-LRR protein encoding accessions were reported as rice blast resistant (Jiang *et al.*, 2012). It has been stated before, BARI Gom 33 is blast resistant wheat genotype in Bangladesh (DGGW, 17; Personal communication, Dr. NCD Barma, Director, BWMRI). BARI Gom 31 and BARI Gom 32 have a moderate level of blast tolerance. BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29 and BARI Gom 30 are highly susceptible to wheat blast (DGGW, 2017). The nineteen markers (M1-M18, *Piz3*) were used to differentiate resistant and susceptible genotypes. The electrophoresis image showed that eighteen designed markers M1-M18 and *Piz3* amplified both resistant and susceptible genotypes (Data not given; Fig. 9). In 11 markers (M1, M3, M5, M6, M7, M8, M9, M11, M12, M17, and *Piz3*) both dark and diffused DNA band were present (Data not given; Fig. 9). Dark and diffused DNA band might occur due to InDel or SNP variation (Raghavan *et al.*, 2007). Further study is needed to find out the InDel and SNP variation.

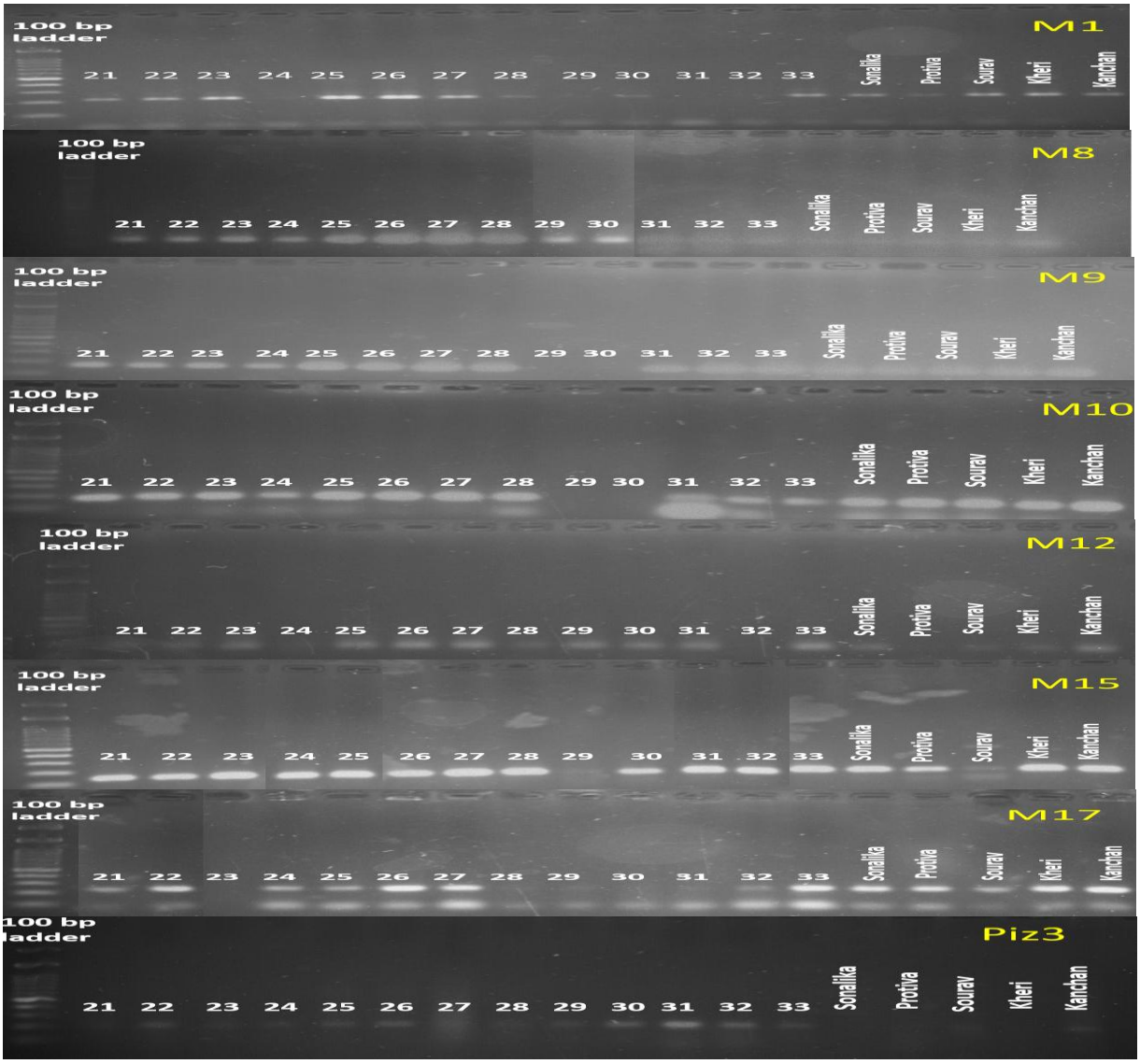

Fig. 9. SSR profile of 18 wheat genotypes respectively, Bari Gom 21, Bari Gom 22, Bari Gom 23, Bari Gom 24, Bari Gom 25, Bari Gom 26, Bari Gom 27, Bari Gom 28, Bari Gom 29, Bari Gom30, Bari Gom 31, Bari Gom 32, Bari Gom 33, Sonalika, Protiva, Sourav, Kheri, Kanchan for M1, M8, M9, M10, M12, M15, M17, and *Piz3* markers.

In essence, disease resistance protein-encoding accessions of *Thatcher* with its paralogs and orthologs of *Pita* gene might bear blast resistance which should be assessed for blast resistance. The nineteen markers could not distinguish contrasting genotypes as blast resistance SSR marker but the dark and diffused DNA band have to study for Insertion/Deletion and single nucleotide polymorphism which may yield functional markers. New NBS-LRR, pathogenesis, CC, LRR, TIR protein domain encoding accessions could to be utilized for developing wheat blast resistance markers.

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