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Phylogenetic Analysis of Blast Resistant RMG7 (Resistance to Magnaporthe Grisea 7) Gene in Cereals (Wheat and Rice)

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Abstract: Understanding the emergence and evolution of plant pathogens has benefited greatly from phylogenetic approaches. Phylogenetic analysis of the Blast Resistant RMG7 (Resistance to Magnaporthe grisea 7) gene would involve the study of its evolutionary relationships with related genes across different species. This study aimed to understand the gene's origin, divergence, and evolutionary history, which can provide insights into its function and potential applications in crop improvement. The study obtained the total of 36 DNA sequences of the RMG7 gene from different organisms (wheat and rice) from publicly available NCBI(https://www.ncbi.nlm.nih.gov) database on 20 May 2023 to investigate its evolution. The Sequences were selected based on the representation of a diverse range of species, including those known to have blast resistance or related traits. Using the generic time reversible model and the maximum likelihood method, evolutionary history was determined. These methods used statistical algorithms to estimate the evolutionary relationships between the sequences, forming a tree that represents their common ancestry. An evolutionary tree evolved using the aligned RMG7 sequences to determine the common ancestor of each strain. The Geneious software, for which purpose (sequence alignment), which tool (such as muscle, geneious aligner, cluster) was used to carry sequence alignment, including parameters (in Geneious: local alignment or global, and at which identity value (65% identical) used in doing the alignment. The phylogenetic analysis showed that the resulting tree to interpret the evolutionary relationships among the RMG7 gene sequences. The Intensified clusters or branches that group sequences with similar blast resistance characteristics or closely related species. Overall, this research provides valuable insights into the evolutionary history, genetic diversity, and relatedness of the RMG7 gene in cereals, particularly in wheat and rice. The findings have implications for understanding the mechanisms of blast resistance and for enhancing crop protection strategies against Magnaporthe grisea.

I. Introduction:

Blast fungi, belonging to the genus Magnaporthe, are a group of devastating plant pathogens that pose a significant threat to global agriculture. These fungi are responsible for causing diseases known as "blast," which affect a wide range of economically important crops, including rice, wheat, barley, and millet. The ability of blast fungi to rapidly adapt and overcome host defenses has made them one of the most destructive groups of plant pathogens worldwide.

Mostly wheat blast thrives in warm and humid conditions, making it particularly problematic in tropical and subtropical regions. Its ability to rapidly adapt and spread has raised concerns about its potential to reach other major wheat-growing areas around the world, including Asia, North America, and Europe, where a crucial main crop is wheat. In contrast to MoT

strains gathered in the early days of observation, when the disease was thought to be a rare occurrence not capable of causing large and recurrent epidemics, it is significant that the strain discovered in Bangladesh conforms to a very aggressive type (ACP Goulart, 1990; Maciel et al., 2014). The majority of wheat cultivars now used for commercial production in South Asia are vulnerable to wheat blast. Although several wheats blast-tolerant or somewhat resistant cultivars have been found in specific geographic locations, no long-lasting resistant cultivar has yet been developed (E Duveiller, 2016). Innovative approaches to management for this disease may be needed due to the the amount of production losses and rapidity of epidemics put on by MoT as well as the absence of resistance.

Blast disease leads to significant yield losses and poses a major threat to global food security. The study estimated the annual potential loss of wheat across the countries that were investigated (India, Pakistan, and Bangladesh) to be 0.89-1.77 million tones, equivalent to USD 132-264 million, assuming a conservative scenario of 5-10% for blast-induced wheat crop loss (Mottaleb et a l., 2018). It is mostly a disease of the wheat head, and under ideal disease conditions, it can result in yield losses of up to 100% (Islam et al., 2020).

Therefore, developing and deploying appropriate fungicides to ensure that farmers have access to efficient substances at the right time and at an affordable price, especially in conditions that are conducive to wheat blast. A concentrated effort and long-term secured investments are required right away to develop and spread blast-resistant wheat cultivars and ensure the public's access to wheat (Mottaleb et a l., 2018).

An integrated management plan should be used to treat this dangerous wheat disease due to limited fungicide efficiency against the condition and a lack of resistant types. In addition, a deeper comprehension of the fungus' biology of infection and its interactions with wheat plants at the molecular and tissue levels is necessary for enhancing disease treatment. Additionally, employing resistance genes like 2NS translocation, Rmg8, and RmgGR119 or sophisticated genomic techniques like CRISPR-Cas, breeding for resistance to wheat blast can be expedited (Islam et al., 2020).

The optimum disease management approach for wheat blast disease should integrate cultivar resistance with suitable agronomic practises, just like it did for rice blast disease. The discovery of wheat blast fungal resistance genes is a crucial first step in creating a disease management strategy (Urashima et al., 2004) . To date, few resistance genes have been tested for subsequent alellisms up to this point. More knowledge of variability in disease response and its inheritance is needed in order to efficiently breed for blast resistance in host crop species (Inukai et al., 2006), as well as the blast fungus's matching avirulence genes' structure and stability (Chuma et al., 2011).

However, the tested germplasms still only contain a small number of sources or resistance genes. Ten distinct genes and a chromosomal fragment have been found to be sources of resistance to the wheat blast fungus thus far. One of these, the Rmg7 gene, which is expressed at the heading stage, was found in an accession of Triticum dicoccum (Tagle et al., 2015).

Another report by(Islam et al., 2019) mentioned that understanding the biology and epidemiology of wheat blast, along with developing effective management strategies, is crucial in mitigating its impact on global wheat production. Ongoing research and collaboration among scientists, breeders, and policymakers are essential to combat this devastating disease and ensure global food security in the face of emerging plant pathogens.

Understanding the genetic basis of resistance and pathotype diversity within a pathogen population, such as the RMG7 gene, is crucial for designing a resistant breeding strategy against the pathogen (Hossain, 2022; Nizolli et al., 2023)

However, lack of efforts to combat wheat blast have been underway, including the development of resistant wheat varieties and the implementation of agronomic practices aimed at minimizing disease incidence. Nevertheless, the rapid evolution of the fungus and its ability to overcome resistance mechanisms have posed challenges in effectively controlling the disease.

In this regard over the years, advancements in molecular biology techniques, particularly DNA sequencing technologies, have revolutionized phylogenetic analysis. By sequencing specific genomic regions or entire genomes of blast fungi, researchers can reconstruct their evolutionary history and unravel the relationships between different species, subspecies, and strains.

Phylogenetic studies of blast fungi have provided valuable insights into their population structure, geographic distribution, and host specificity. These analyses have revealed the existence of distinct lineages within the Magnaporthe genus, each associated with specific host plants or geographic regions. Such information is essential for developing effective disease management strategies, including the deployment of resistant crop varieties and the implementation of targeted control measures.

Phylogenetic analysis of the Blast Resistant RMG7 (Resistance to Magnaporthe grisea 7) gene involves investigating the evolutionary relationships and patterns of this gene across different species. By studying the gene's evolutionary history,

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researchers can gain insights into its origin, diversification, and potential functional implications for blast resistance in crops.

Furthermore, phylogenetic analysis has shed light on the mechanisms of genetic variation and adaptation in blast fungi. It has helped identify genetic factors responsible for pathogenicity, virulence, and the ability of blast fungi to overcome host resistance. Understanding the evolutionary processes driving these traits can inform breeding efforts to develop durable resistance in crop plants.

Phylogenetic analysis, the study of evolutionary relationships among organisms based on their genetic information, has played a crucial role in understanding the diversity and evolution of blast fungi. By examining the genetic makeup of different blast fungal strains, researchers can gain insights into the origin, dispersal patterns, and genetic diversity within the genus Magnaporthe.

In summary, phylogenetic analysis has proven to be a powerful tool for investigating the evolutionary history and genetic diversity of blast fungi. The insights gained from these studies have important implications for understanding the emergence and spread of blast diseases and developing effective strategies to combat them. This analysis helps to elucidate the gene's evolutionary dynamics, such as gene duplications, functional diversification, or adaptations that may have contributed to blast resistance.

Furthermore, the study identified novel RMG7 gene variants that have not been previously reported, expanding our understanding of the genetic diversity within this blast resistance gene in cereals. The identification and characterization of these novel variants contribute to the development of improved blast-resistant cultivars through marker-assisted breeding and genetic engineering approaches. Continued research in this area is crucial for safeguarding global food security and mitigating the impact of blast fungi on agricultural systems.

II. Materials and Method:

This section explains the methodological approaches, process, techniques, and tools used to conduct this study and show how all the stated key objectives of this study have been achieved. In accomplishing the research aim, this section also explains the different steps and activities that is involved in achieving the objective of the study. The steps involved in conducting a phylogenetic analysis of the RMG7 gene include Sequence Data Retrieval, Sequence Alignment, Tree construction, and subsequent Evolutionary analysis of the resulting phylogenetic tree.

Sequence Data Retrieval:

Obtain the total of 36 DNA sequences (Table 1) of the RMG7 gene for two different organisms (wheat and rice) from publicly available database NCBI (https://www.ncbi.nlm.nih.gov) on 20 May 2023 to investigate its evolution. The Sequences were selected based on the representation of a diverse range of species, including those known to have blast resistance or related traits.

TABLE 1. Characteristics of the 36 DNA sequences used in this study

Accession number	Organism name	Strain	GC content
KM004023.1	Pyricularia grisea	AvrPi9	48.80%
AF207841.1	Pyricularia grisea	AVR-Pita	36.80%
AY245425.1	Pyricularia grisea	CHW (cytb)	26.20%
EU837058.1	Magnaporthe grisea	81278ZB15 (AvrPiz-t)	46.80%
AY245427.1	Magnaporthe grisea	GG3H 5-1 cytochrome b (cytb)	26.40%
AY245424.1	Magnaporthe grisea	Guy11	26.10%
AY245426.1	Magnaporthe grisea	LpMD01-1 cytochrome b (cytb)	26.70%
XM_003718942.1	Pyricularia oryzae	MGG_00141	54.60%
XM_003718818.1	Pyricularia oryzae	MGG_00239	63.70%
XM_003718572.1	Pyricularia oryzae	MGG_00447	52.40%
XM_003714435.1	Pyricularia oryzae	MGG_01511	63.20%
XM_003714516.1	Pyricularia oryzae	MGG_01586	54.40%
XM_003708785.1	Pyricularia oryzae	MGG_02069	53.30%
XM_003709070.1	Pyricularia oryzae	MGG_02348	62.10%
XM_003710582.1	Pyricularia oryzae	MGG_05723	56.40%
XM_003710614.1	Pyricularia oryzae	MGG_05746	52.70%

XM_003709463.1	Pyricularia oryzae	MGG_06794	60.40%
XM_003711293.1	Pyricularia oryzae	MGG_07444	54.00%
XM_003713034.1	Pyricularia oryzae	MGG_07848	55.70%
XM_003714964.1	Pyricularia oryzae	MGG_08060	53.90%
XM_003713960.1	Pyricularia oryzae	MGG_08970	63.70%
XM_003709732.1	Pyricularia oryzae	MGG_09171	54.80%
XM_003712194.1	Pyricularia oryzae	MGG_09502	50.50%
XM_003712126.1	Pyricularia oryzae	MGG_09566	54.90%
XM_003709929.1	Pyricularia oryzae	MGG_09884	53.60%
XM_003713736.1	Pyricularia oryzae	MGG_10147	54.10%
XM_003719380.1	Pyricularia oryzae	MGG_10410	57.60%
XM_003710027.1	Pyricularia oryzae	MGG_10646	52.90%
XM_003721027.1	Pyricularia oryzae	MGG_12612	52.90%
XM_003716997.1	Pyricularia oryzae	MGG_12817	52.70%
XM_003710731.1	Pyricularia oryzae	MGG_13762	55.10%
XM_003714275.1	Pyricularia oryzae	MGG_14778	58.20%
XM_003714879.1	Pyricularia oryzae	MGG_15334	54.00%

Sequence Alignment:

Sequence alignment of the Rmg7 gene involves comparing the nucleotide or amino acid sequence of the gene from different rice and wheat varieties or accessions to identify similarities and differences. There are multiple processes involved in performing a sequence alignment of the Rmg7 gene from various strains of two different cereals. By comparing and contrasting DNA sequences, sequence alignment can reveal information about the evolutionary links or functional conservation of the gene. The retrieved data was then uploaded to the Geneious software for sequence alignment and a sequence alignment tool (such as muscle, geneious aligner, or cluster) was used for the alignment.

Parameters, including the choice between local alignment or global alignment, and the specified identity value (e.g., 65% identical), were configured in Geneious during the alignment process. An appropriate sequence alignment method was selected based on the type of sequence (DNA) and the expected level of conservation in the Rmg7 gene. The alignment algorithm was employed to compare the sequences and identify regions of similarity or conservation.

Tree construction and Evolutionary analysis:

A phylogenetic tree was constructed using the aligned RMG7 sequences to determine the common ancestor of each strain and to infer the evolutionary relationships. The evolutionary history was inferred using the maximum likelihood method based on the general time reversible model. This method utilized the aligned sequences and the selected evolutionary model to estimate the phylogenetic tree. This method also used statistical algorithms to estimate the evolutionary relationships between the sequences, forming a tree that represents their common ancestry. The constructed phylogenetic tree was visualized using the Geneious software.

After sequence alignment, we carried phylogenetic analysis using temarua as the genetic distant model, and **Neighbor-Joining** with a bootstrap value of 1000 and 100 numbers of replicates to indicate the revolutionary process analyzed over time, edited with Geneious tree builder version 9.0.5 to determine the evolutionary relatedness and diversities. Comparative analysis with a distance matrix of the tree was performed on Geneious (https://www.geneious.com) based on statistical analysis to determine positions of significant difference between the samples. The generated alignment was examined to identify conserved regions, insertions, deletions, and variations among the sequences. Conserved regions indicate important functional elements of the gene, while variations may represent potential differences in disease resistance.

III. Results:

a) Alignment View:

The output of the alignment was a multiple sequence alignment (MSA), which shows the aligned sequences with gaps introduced to maximize the similarity.

The consensus identity view is shown in Fig. 1.

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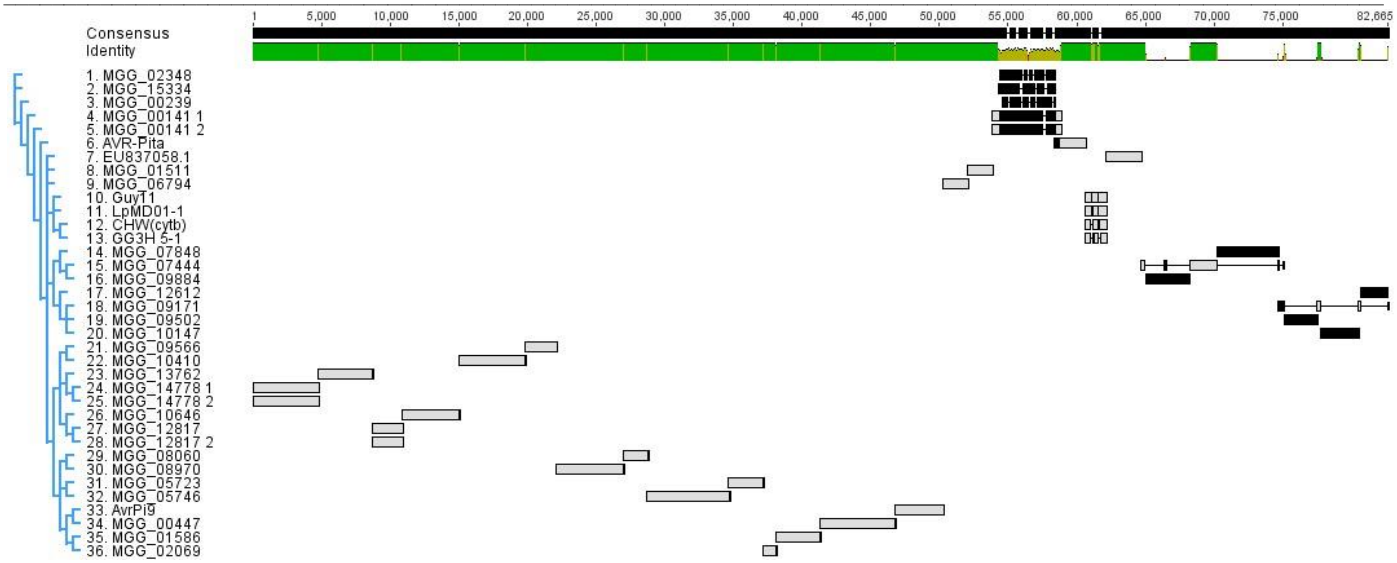

Fig. 1. Showed consensus similarities and variants among different strains from two organism Wheat, and Rice. The consensus identity view of RMG7 gene with 55-60% similarity

Multiple sequence alignment using CLUSTALW (https://www.ebi.ac.uk/Tools/services/rest/muscle), showed that all the genomes that formed a clade with the strain from wheat and Rice generally had more than 70% similarity in the genetic sequence. Sequences in the alignment were compared to the consensus to identify polymorphisms. At each position, the consensus is the allele with frequency >50%. N is ambiguity if no allele exceeds 50%.

b) Sequence alignment text format

The sequences are represented using single-letter codes for nucleotides (A, T, G, C). Multiple sequences can be aligned and represented in the same format, with each sequence starting with its own identifier.

```
                1         10        20        30        40        50        60
                |         |         |         |         |         |         |
      AvrPi9    ------------------------------------------------------------
     AVR-Pita   ------------------------------------------------------------
          CHW   ------------------------------------------------------------
    EU837058.1  ------------------------------------------------------------
     GG3H 5-1   ------------------------------------------------------------
        Guy11   ------------------------------------------------------------
     LpMD01-1   ------------------------------------------------------------
   MGG_00141 1  ------------------------------------------------------------
   MGG_00141 2  ------------------------------------------------------------
     MGG_00239  ------------------------------------------------------------
     MGG_00447  ------------------------------------------------------------
     MGG_01511  ------------------------------------------------------------
     MGG_01586  ------------------------------------------------------------
     MGG_02069  ------------------------------------------------------------
     MGG_02348  ------------------------------------------------------------
```

```
MGG_05723   ------------------------------------------------------------
MGG_05746   ------------------------------------------------------------
MGG_06794   ------------------------------------------------------------
MGG_07444   ------------------------------------------------------------
MGG_07848   ------------------------------------------------------------
MGG_08060   ------------------------------------------------------------
MGG_08970   ------------------------------------------------------------
MGG_09171   ------------------------------------------------------------
MGG_09502   ------------------------------------------------------------
MGG_09566   ------------------------------------------------------------
MGG_09884   ------------------------------------------------------------
MGG_10147   ------------------------------------------------------------
MGG_10410   ------------------------------------------------------------
MGG_10646   ------------------------------------------------------------
MGG_12612   ------------------------------------------------------------
MGG_12817 1 ------------------------------------------------------------
MGG_12817 2 ------------------------------------------------------------
MGG_13762   ------------------------------------------------------------
MGG_14778 1 GCTCCTTTAAGCTCAGCAAACCACGTCGTGGCCATTTGAAGCAAAACAACCAAGCCACAC
MGG_14778 2 GCTCCTTTAAGCTCAGCAAACCACGTCGTGGCCATTTGAAGCAAAACAACCAAGCCACAC
MGG_15334   ------------------------------------------------------------

AvrPi9      ------------------------------------------------------------
AVR-Pita    ------------------------------------------------------------
CHW         ------------------------------------------------------------
EU837058.1  ------------------------------------------------------------
GG3H 5-1    ------------------------------------------------------------
Guy11       ------------------------------------------------------------
LpMD01-1    ------------------------------------------------------------
MGG_00141 1 ------------------------------------------------------------
MGG_00141 2 ------------------------------------------------------------
MGG_00239   ------------------------------------------------------------
MGG_00447   ------------------------------------------------------------
MGG_01511   ------------------------------------------------------------
MGG_01586   ------------------------------------------------------------
MGG_02069   ------------------------------------------------------------
```

```
   MGG_02348   ------------------------------------------------------------
   MGG_05723   ------------------------------------------------------------
   MGG_05746   ------------------------------------------------------------
   MGG_06794   ------------------------------------------------------------
   MGG_07444   ------------------------------------------------------------
   MGG_07848   ------------------------------------------------------------
   MGG_08060   ------------------------------------------------------------
   MGG_08970   ------------------------------------------------------------
   MGG_09171   ------------------------------------------------------------
   MGG_09502   ------------------------------------------------------------
   MGG_09566   ------------------------------------------------------------
   MGG_09884   ------------------------------------------------------------
   MGG_10147   ------------------------------------------------------------
   MGG_10410   ------------------------------------------------------------
   MGG_10646   ------------------------------------------------------------
   MGG_12612   ------------------------------------------------------------
 MGG_12817 1   ------------------------------------------------------------
 MGG_12817 2   ------------------------------------------------------------
   MGG_13762   ------------------------------------------------------------
 MGG_14778 1   CAAGATGGAAGCCTCCAATAATTCGTGACCAATTTTGAAGATCTTTCAAGATAAGGAATC
 MGG_14778 2   CAAGATGGAAGCCTCCAATAATTCGTGACCAATTTTGAAGATCTTTCAAGATAAGGAATC
   MGG_15334   ------------------------------------------------------------

      AvrPi9   ------------------------------------------------------------
    AVR-Pita   ------------------------------------------------------------
         CHW   ------------------------------------------------------------
  EU837058.1   ------------------------------------------------------------
    GG3H 5-1   ------------------------------------------------------------
       Guy11   ------------------------------------------------------------
    LpMD01-1   ------------------------------------------------------------
 MGG_00141 1   ------------------------------------------------------------
 MGG_00141 2   ------------------------------------------------------------
   MGG_00239   ------------------------------------------------------------
   MGG_00447   ------------------------------------------------------------
   MGG_01511   ------------------------------------------------------------
   MGG_01586   ------------------------------------------------------------
```

```
   MGG_02069   ------------------------------------------------------------
   MGG_02348   ------------------------------------------------------------
   MGG_05723   ------------------------------------------------------------
   MGG_05746   ------------------------------------------------------------
   MGG_06794   ------------------------------------------------------------
   MGG_07444   ------------------------------------------------------------
   MGG_07848   ------------------------------------------------------------
   MGG_08060   ------------------------------------------------------------
   MGG_08970   ------------------------------------------------------------
   MGG_09171   ------------------------------------------------------------
   MGG_09502   ------------------------------------------------------------
   MGG_09566   ------------------------------------------------------------
   MGG_09884   ------------------------------------------------------------
   MGG_10147   ------------------------------------------------------------
   MGG_10410   ------------------------------------------------------------
   MGG_10646   ------------------------------------------------------------
   MGG_12612   ------------------------------------------------------------
 MGG_12817 1   ------------------------------------------------------------
 MGG_12817 2   ------------------------------------------------------------
   MGG_13762   ------------------------------------------------------------
 MGG_14778 1   AAAATCCATGTCTCCTTGATTATATGGATTGAGGGGGGAGTTTTCAAGGGTATTCGTCAT
 MGG_14778 2   AAAATCCATGTCTCCTTGATTATATGGATTGAGGGGGGAGTTTTCAAGGGTATTCGTCAT
   MGG_15334   ------------------------------------------------------------

      AvrPi9   ------------------------------------------------------------
    AVR-Pita   ------------------------------------------------------------
         CHW   ------------------------------------------------------------
  EU837058.1   ------------------------------------------------------------
    GG3H 5-1   ------------------------------------------------------------
       Guy11   ------------------------------------------------------------
    LpMD01-1   ------------------------------------------------------------
 MGG_00141 1   ------------------------------------------------------------
 MGG_00141 2   ------------------------------------------------------------
   MGG_00239   ------------------------------------------------------------
   MGG_00447   ------------------------------------------------------------
   MGG_01511   ------------------------------------------------------------
```

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```
MGG_01586    ------------------------------------------------------------
MGG_02069    ------------------------------------------------------------
MGG_02348    ------------------------------------------------------------
MGG_05723    ------------------------------------------------------------
MGG_05746    ------------------------------------------------------------
MGG_06794    ------------------------------------------------------------
MGG_07444    ------------------------------------------------------------
MGG_07848    ------------------------------------------------------------
MGG_08060    ------------------------------------------------------------
MGG_08970    ------------------------------------------------------------
MGG_09171    ------------------------------------------------------------
MGG_09502    ------------------------------------------------------------
MGG_09566    ------------------------------------------------------------
MGG_09884    ------------------------------------------------------------
MGG_10147    ------------------------------------------------------------
MGG_10410    ------------------------------------------------------------
MGG_10646    ------------------------------------------------------------
MGG_12612    ------------------------------------------------------------
MGG_12817 1  ------------------------------------------------------------
MGG_12817 2  ------------------------------------------------------------
MGG_13762    ------------------------------------------------------------
MGG_14778 1  GAACGAATAACTCGGCGTCCGCGAACAGCGTGCTTCTTGGCTTGCGCAAAAAAAAGACCC
MGG_14778 2  GAACGAATAACTCGGCGTCCGCGAACAGCGTGCTTCTTGGCTTGCGCAAAAAAAAGACCC
MGG_15334    ------------------------------------------------------------
```

Fig. 2. Showed Sequence alignment in text format.

c) Phylogenetic tree

The maximum likelihood tree is shown in Fig. 3.

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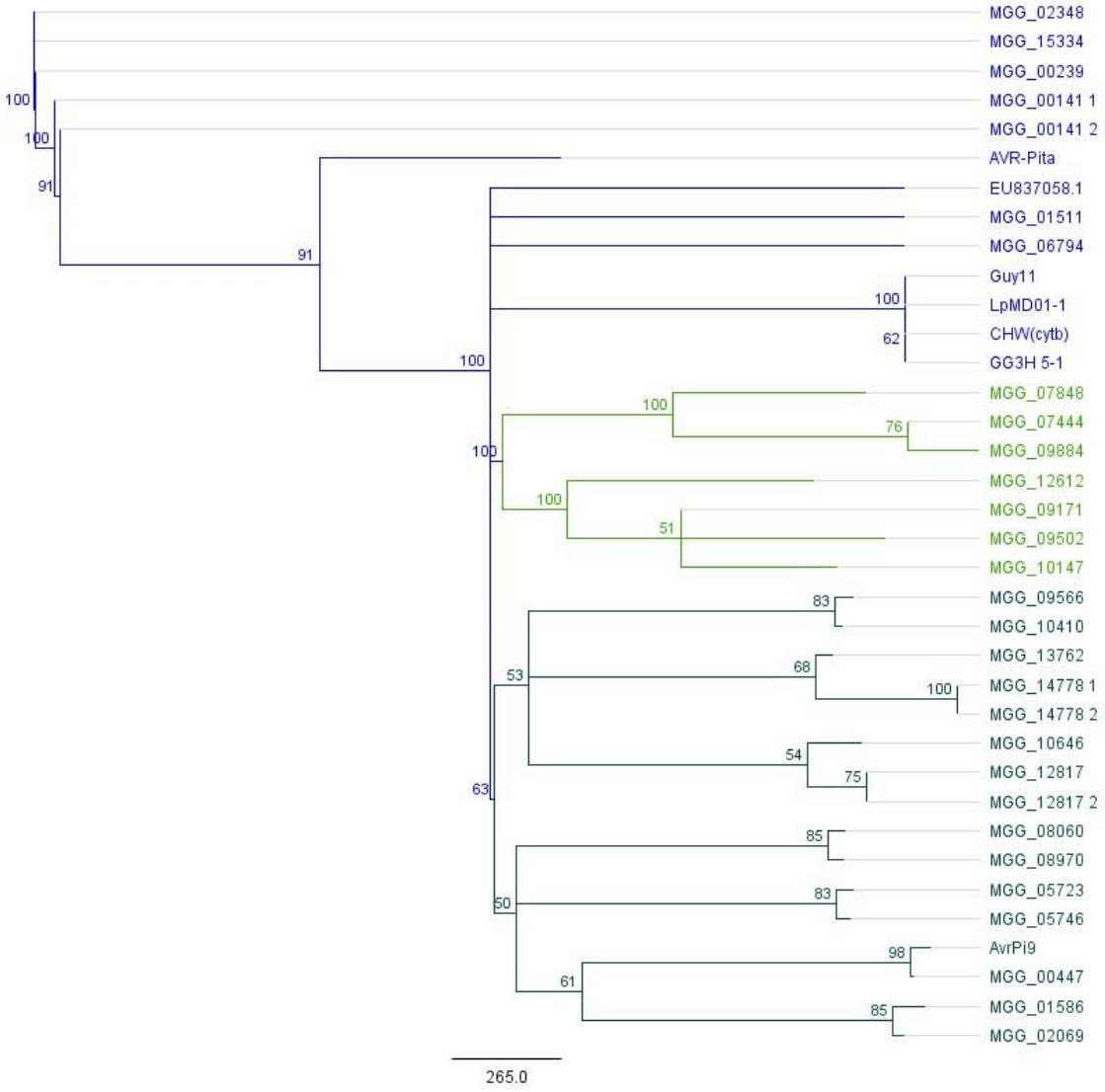
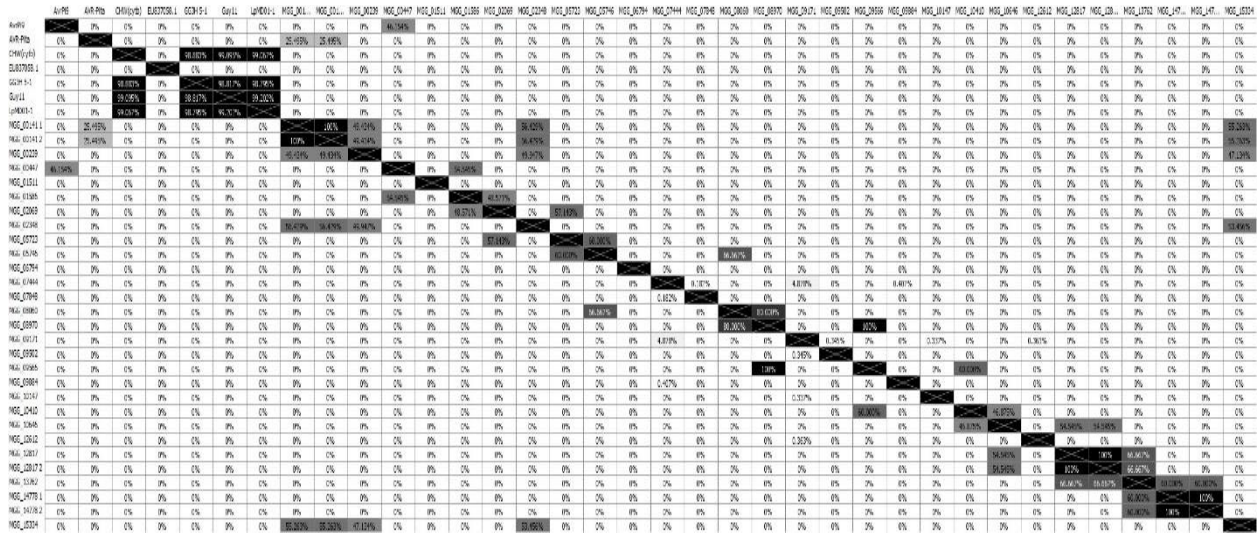


FIG. 3. Maximum Likelihood Tree of RMG7 gene. Sequences were aligned using geneious aligner and tree construction using Geneious tree builder version 9.0.5.

d) Distance Matric / Heatmap



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Discussion:

To interpret the genetic diversity of the organisms listed in the phylogenetic analysis, we can consider several factors such as the organism names, strains, and GC content. The branches indicate the genetic distance or divergence between the organisms. In other words, the length and structure of the branches show how closely or distantly related the species or strains within a cluster. The bootstrap value associated with each branch represents the statistical support for that particular grouping or relationship. It is a measure of confidence in the accuracy of the branch placement. Higher bootstrap values (typically ranging from 0 to 100) indicate greater confidence in the grouping or relationship being depicted by the branch. Therefore, in our phylogenetic tree, the presence of branches with diverse bootstrap values suggests that there is genetic diversity between the two different species and strains within species. Some branches may have high bootstrap values, indicating a strong genetic relationship, while others may have lower bootstrap values, suggesting a weaker genetic relationship. Here are some observations.

In the phylogenetic tree in Fig.3, three distinct clusters have been identified. Each of these clusters exhibits branches with diverse bootstrap values, which illustrate the genetic diversity present between two different species and strains within those species. The organisms in the analysis belong to two main species: Pyricularia oryzae and Magnaporthe grisea. Within each species, there are different strains identified. In our study, the phylogenetic tree contains three distinct clusters identified by three different colors such as cluster-1, cluster-2, cluster-3 which means that the organisms or species being compared can be grouped into three major categories based on their genetic similarities.

Each cluster in the phylogenetic tree is represented by branches, which illustrate the evolutionary relationships between two different organisms within that cluster. Cluster-1 comprises two different genera: wheat (Triticum) and rice (Oryza), as well as several strains with specific identifiers such as MGG_09566, MGG_10410, MGG_13762, MGG_14778, MGG_10646, MGG_12817, MGG_12817 2, MGG_08060, MGG_08970, MGG_05723, MGG_05746, AvrPi9, MGG_00447, MGG_01586, and MGG_02069. In this case, a bootstrap value of 85 between two strains MGG_01586, & MGG_02069 indicates a relatively high level of support for the relationship between these two strains. It could indicate that these two strains are closely related or share a recent common ancestor.

The other two strains in this cluster, AvrPi9 & MGG_00447, from the species wheat and rice, respectively, shared a bootstrap value of 98 in a phylogenetic tree, which suggests a strong statistical support for their close relationship or shared ancestry. The high bootstrap value of 98 indicates a strong statistical support for the close relationship between the wheat strain AvrPi9 and the rice strain MGG_00447 in the phylogenetic tree. We can infer that there is a reasonably strong statistical support for the grouping of these four strains in a shared branch or clade within the phylogenetic tree that have 39% genetic diversity.

MGG_05723 & MGG_05746 are more closely related to each other and MGG_08060 & MGG_08970 shared a more recent ancestor. Hence MGG_05723 & MGG_05746, MGG_08060 & MGG_08970 are sister taxa of AvrPi9 & MGG_00447 and MGG_01586, & MGG_02069 that showed genetic diversity 50%.

The strains MGG_10646 and MGG_12817 exhibit 46% diversity, it suggests that they have a relatively high level of genetic variation between them. This means that approximately 46% of their genetic sequences or markers differ from each other. Whereas the strains MGG_13762 and MGG_14778 have a bootstrap value of 68 in a phylogenetic tree, it indicates a moderate level of support for their relationship or grouping within the tree. MGG_09566 & MGG_10410 are more closely related to each other.

Cluster-2 that includes several strains from only one specie rice with specific identifiers such as MGG_10147, MGG_09502, MGG_09171, MGG_12612, MGG_09884, MGG_70444, MGG_07848. The three strains MGG_10147, MGG_09502, and MGG_09171 exhibit a genetic diversity of 49% in a phylogenetic tree, it indicates a relatively high level of variation among these strains. This means that approximately 49% of their genetic sequences or markers differ from each other. MGG_12612 are sister taxa of these three strains. MGG_70444 and MGG_09884 are more closely related to each other. MGG_07848 shared a more recent ancestor with MGG_70444 & MGG_09884 that are 100% identical.

Cluster-3 of the above phylogenetic tree with strains from wheat and rice, represented by specific identifiers like GG3H 5-1, CHW (cytb), LpMD01-1, Guy11, MGG_06794, MGG_01511, EU837058.1, AVR-Pita, MGG_00141, MGG_00239, MGG_15334, and MGG_02348, can be understood by examining the genetic variations and relationships among these strains. The strains GG3H 5-1 and CHW (cytb) exhibit a genetic divergence of 38%, it indicates a relatively moderate level of genetic variation between these two strains those are also 100% identical to both LpMD01-& Guy11. These four identifiers are also quite similar to three additional strains, including MGG_06794, MGG_01511, and EU837058.1. Whereas the strain AVR-Pita is closely related to those strains as explained. The rest of the five strains in the cluster-3 are MGG_00141, MGG_00239, MGG_15334, and MGG_02348 that shared a common ancestor and exhibit no variations, it suggests that they are highly similar or identical in the specific genetic elements analyzed.

In summary, it appears that Cluster-2 and Cluster-3 showed more similarity or identity to each other, while Cluster-1 exhibits more divergence compared to the other groups. A high bootstrap value like 98 indicates that there is strong evidence supporting the close relationship or shared ancestry between AvrPi9 and MGG_00447. The close relationship between AvrPi9 and MGG_00447 could have implications for their genetic similarity, functional characteristics, or evolutionary history. It may indicate shared genetic traits, conserved sequences, or common evolutionary pressures.

IV. Conclusion:

Overall, the genetic diversity in this phylogenetic analysis can be inferred from the presence of different strains within the same species, as well as the variations in GC content. The diversity in strains and GC content indicates the presence of genetic variation and potentially different genetic characteristics among the organisms.

For Pyricularia oryzae, we have strains such as AvrPi9, AVR-Pita, and CHW (cytb). For Magnaporthe grisea, we have strains like 81278ZB15 (AvrPiz-t), GG3H 5-1 cytochrome b (cytb), Guy11, and LpMD01-1 cytochrome b (cytb). These strains indicate genetic variation within the species, potentially resulting in different phenotypic traits. The GC content is a measure of the proportion of guanine (G) and cytosine (C) bases in the DNA sequence. It is often used as an indicator of genetic diversity. In this analysis, we can observe variations in the GC content among the different strains. For example, Pyricularia oryzae strain MGG_00239 has a GC content of 63.70%, while strain MGG_08970 has a GC content of 63.70%. This variation in GC content suggests genetic diversity among the strains.

It is also worth noting the presence of "AvrPi9" in the list of identifiers. "AvrPi9" could potentially refer to a gene or genetic element that plays a role in pathogen recognition or plant defense mechanisms. The identifier AVR-Pita refers to a specific avirulence gene (Avr-Pita) in the rice blast fungus Magnaporthe oryzae. Avr-Pita triggers a defense response in rice plants carrying the corresponding resistance gene Pita.

The findings from a phylogenetic analysis of the Blast Resistant RMG7 gene can have significant implications for crop improvement. Understanding the gene's evolutionary history and relationships with other blast resistance genes can aid in the development of strategies to enhance crop resistance against Magnaporthe grisea. By identifying closely related genes with similar functions, researchers can potentially transfer blast resistance traits across different crop species through breeding or genetic engineering approaches.

This analysis enables researchers to identify patterns of relatedness, group gene strains with similar blast resistance characteristics, and infer functional similarities or differences based on evolutionary relationships and gain insights into the evolutionary processes that have shaped the gene's blast resistance function.

In conclusion, phylogenetic analysis of the Blast Resistant RMG7 gene provides a powerful tool for understanding its evolutionary history and functional implications in blast resistance. This analysis contributes to our knowledge of the gene's origin, diversification, and potential applications for crop improvement, ultimately aiding in the development of strategies to mitigate the impact of blast disease on agricultural productivity.

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