

Original Research Article

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Comparative Analysis of Orthologous Genes between *Anguina tritici*, *Ditylenchus destructor* and *Meloidogyne incognita*

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ABSTRACT

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Nematodes are the most abundant metazoans and highly diverse playing significant role in nutrient recycling, successful bioagent of insect pest and most importantly yield loss to crop. The model nematode (*Caenorhabditis elegans*) was first nematode which genome sequenced in 1998 and given impetus for future endeavor to generate genomic information for other nematodes particularly for PPNs. Comparative genomics is one of the approaches to predict the genes shared with different species and also to unravel the genes unique and novel to a particular species. In the present study we have used a graph-based method by utilizing Orthovenn 2 tool for orthologous analysis to compare and annotate the orthologous cluster between the genome of *Anguina tritici*, *Ditylenchus destructor* and *Meloidogyne incognita*.

Introduction

Nematodes are the most abundant metazoans and highly diverse affecting human, animal and plant health and some are playing significant role in nutrient recycling and as successful bioagent of insect pest (Blaxter, 2003; Lamshead, 1993; Andrassy and Zombori, 1976). Among all the nematodes plant parasitic nematodes (PPNs) occupy significant posing due to their role in damaging crop and inflicting yield loss to wide range of crops (Singh *et al.*, 2015).

Nematodes are very distinct organism due to its adaptability in diverse climatic condition and has been attributed due to morphological, behavioural and genetic plasticity (Coghlan, 2005).

The model nematode (*Caenorhabditis elegans*) was first nematode which genome sequenced in 1998 and given impetus for future endeavor to generate genomic information for other nematodes particularly for PPNs. Since then more than 100s of nematode genome have been sequenced and

interpreted for diverse biological complexity and mystery of evolution. Related to evolutionary history of nematode that our understanding about the evolution of nematodes is enriching due to availability of genome sequences of wide range of nematodes including free living to parasite of plants (<https://parasite.wormbase.org/index.html>).

The available genomic datasets are ideal to investigate in depth about diverse biological phenomenon through comparative genomics tool. Comparative genomics among different feeding group of nematodes are important to unravel the force enabling them to feed on different parts of the plant despite their similar morphological architect. The mystery behind host suitability to adaptability to feed on different plant part, survivability lies in the genetic trait of nematode species. Although the genomic data are available on large scale on different database but analysing, investigating and drawing meaningful conclusion is still a challenge.

Comparative genomics is one of the approaches to predict the genes shared with different species and also to unravel the genes unique and novel to a particular species. The homologous genes (orthologue and parologue) related to speciation or duplication are indispensable in tracing the evolutionary pattern of an organism and furthermore inferring orthology is most accurate alternative to describe similarities and differences in a genome (Gabaldon, 2013). Orthologs gene generally shows their syntenic relationship with closely related species and can be traced to a Common ancestor during speciation events.

In contrast to orthologs, Paralogs show limited synteny, divergence in speciation and originated from duplication events in a species (Altenhoff and Dessimoz, 2012). The

presence of similar orthologous sequences in different species indicates their similar biological function and contrary if sequence show greater divergence then other species may perform distinct function in particular species (Fitch, 2000).

In comparative genomics of two species, identification of orthologous genes and their degree of similarity between two species are the major steps in understanding evolutionary pattern of genes and genomes (Koonin *et al.*, 1996). In the present study sequenced genome of seed gall nematode (*A. tritici*) and in its comparative genomics study enabled us for identification of orthologues genes, their function and evolution among different species of nematodes. In the present study we have used a graph-based method by utilizing Orthovenn 2 tool (Xu *et al.*, 2019) for orthologous analysis to compare and annotate the orthologous cluster between the genome of *Anguina tritici*, *Ditylenchus destructor* and *Meloidogyne incognita*.

Materials and Methods

Genome sequencing and assembly

Recently we sequenced and assembled the draft genome of *Anguina tritici* (The gene bank submission will be available soon) using next generation sequencing approach. The obtained genomic data were screened for quality check and assembled using Abyss programme.

Retrieving protein dataset of nematodes

Protein sequence of *A. tritici* were predicted from de novo sequencing and assembly of genome using Trans Decoderprogramme (Haas *et al.*, 2013). Protein sequence of *M. incognita* and *D. destructor* were downloaded from NCBI database (Zheng *et al.*, 2016; Abad *et al.*, 2008).

Genome comparison between *Anguina tritici*, *Ditylenchus destructor* and *Meloidogyne incognita*

Genome of three nematode species namely *A. tritici*, *D. destructor* and *M. incognita* were compared using OrthoVenn 2.0 web server based on DIAMOND (v0.9.24) all against all comparison of nematode protein sequences. The orthologue clustering programme uses most popular heuristic best match approach for identification of orthologous genes conserved between two species. The programme was run after uploading each nematode protein sequences on web server with default parameter (with e-value 1e-5 and inflation value of 1.5) extended with pairwise heatmap. The obtained result can be accessed at supporting link 3

Motif and phylogenetic analysis of Pectatelyase gene

Annotated orthologue clusters using amigo gene ontology (Carbon *et al.*, 2009) revealed various function among which the cluster 263 showed Pectatelyase activity in all three nematodes. Cluster 263 were selected for motif analysis using MEME version 4.12.0 (Bailey *et al.*, 2009) and the protein hits were aligned using MUSCLE (Edgar *et al.*, 2004).

Results and Discussion

In the recent past, genome sequencing efforts for nematodes have generated the huge amount of genomic dataset. Their comparative genomic analysis has revealed the origin of large number of genes through horizontal gene transfer events from bacteria have enabled the tylenchid nematode to parasitize plants (Mitreva *et al.*, 2005). Therefore, genomic resources have been proven to be a potential resource for understanding of molecular mechanism, relations and evolutionary dynamics with

other nematodes as well organism. In our effort to comparative genomics of *A. tritici* with other two nematodes hypothesized to find conserved and diverse genes among *A. tritici*, *D. destructor* and *M. incognita*.

Orthologous cluster analysis of three nematode species (*A. tritici*, *D. destructor* and *M. incognita*) using OrthoVenn 2.0 web server displayed comparison results in cluster table (Fig. 1 A, supporting link 1). The predicted summarized clustering result indicates column as species while each row of cell graphs an orthologous cluster group. The programme predicted the presence of 12 number of cluster group (4 in each nematode species) and absence of cluster group in 9 cells (Fig. 1 A). The cumulative number of protein sequences predicted to be involved in cluster group of each nematode species were varied from 420-3453 (bar plot stacked at right in Fig. 1 A). The *A. tritici* and *M. incognita* revealed to have low number of protein sequences (420) involved in cluster group in comparison to the *A. tritici* and *D. destructor*. OrthoVenn displayed Venn diagram indicating the number of orthologues shared between the three species in which 3453 clusters were found to be shared between *A. tritici*, *D. destructor* and *M. incognita*. The obtained summary result suggested that a total of 22989 proteins, 6171 clusters and 10625 singletons were found in *A. tritici* which were to be highest in comparison to the other nematode species (Fig. 1 B).

The number of orthologue cluster shared between *A. tritici* and *D. destructor* were found to be highest (1726) than *D. destructor* vs *M. incognita* (764) and *A. tritici* vs *M. incognita* (420). Additionally, the Bar plot revealed the presence of total number of clusters were 6171, 6523 and 6301 respectively for *A. tritici*, *D. destructor* and *M. incognita* (Fig. 1 C). Pair wise heat map

analysis revealed the overlapping cluster information in pairwise fashion among all the three-nematode species (Fig. 1 D, Table 1). Orthologues gene cluster shared to all the three nematodes were annotated to know their role in various biological process, molecular function and cellular component (Table 2, 3, 4 and Fig. 2). The details of annotations can be accessed at supporting link 2.

The annotation of clusters shared among all three nematode species revealed to have a suite of large number of genes playing variety of function in biological process such as host finding, parasitism, reproduction and survival. These genes were found to be identical to the *D. destructor* and *M. incognita* in comparison

to the *A. tritici* (Abad *et al.*, 2008, Szitenberg *et al.*, 2017, Zheng *et al.*, 2016). GO analysis reveals the most abundant number of GO were enriched for biological process, metabolic and cellular process (Table 2) and in case of molecular function GO terms significantly abundant for ion binding (Table 2).

Overall GO analysis revealed the largest number of GO terms was associated for biological process, cellular components and molecular functions. Other than shared common clusters there were 572 clusters in *A. tritici*, 580 in *D. destructor* and 1664 in *M. incognita* as unique and specific to their origin (Fig. 1).

Table.1 Indicating the cluster number overlapped in genome of each nematode species in pairwise heat map

<i>Meloidogyne incognita</i>	3873	4217	-----
<i>Ditylenchus destructor</i>	5179	-----	4217
<i>Anguina tritici</i>		5179	3873
	<i>Anguina tritici</i>	<i>Ditylenchus destructor</i>	<i>Meloidogyne incognita</i>

Table.2 Top 10 highest number of accessions for biological process

Slimmed GO	Count of unique input accessions	Name
GO:0008150	1602	biological process
GO:0008152	946	metabolic process
GO:0009987	939	cellular process
GO:0044237	690	cellular metabolic process
GO:0043170	678	macromolecule metabolic process
GO:0065007	657	biological regulation
GO:0044238	528	primary metabolic process
GO:0006807	441	nitrogen compound metabolic process
GO:0050896	413	response to stimulus
GO:0016070	399	RNA metabolic process

Table.3 Top 10 highest number of accessions for Molecular function

Slimmed GO	Count of unique input accessions	Name
GO:0043167	48	ion binding
GO:0003674	41	molecular function
GO:0003676	27	nucleic acid binding
GO:0016787	25	hydrolase activity
GO:0005215	23	transporter activity
GO:0016740	20	transferase activity
GO:0005488	19	binding
GO:0016491	16	oxidoreductase activity
GO:0005515	12	protein binding
GO:0030234	10	enzyme regulator activity

Table.4 Top 10 highest number of accessions for Cellular components

Slimmed GO	Count of unique input accessions	Name
GO:0005575	56	cellular component
GO:0016020	53	membrane
GO:0044464	26	cell part
GO:0005622	18	intracellular
GO:0043226	13	organelle
GO:0005634	10	nucleus
GO:0043229	10	intracellular organelle
GO:0005576	7	extracellular region
GO:0005739	6	mitochondrion
GO:0005856	4	cytoskeleton

Fig.1 (A) Occurrence table indicating shared orthologue groups patterns between *A. tritici*, *D. destructor* and *M. incognita*. Left pattern indicating the species of nematode involved in clusters, shared cluster count among them and protein count among the shared clusters (B) cluster count in genome of each nematode species. Singleton describing those genes having no orthologue; (C) Venn diagram showing the shared orthologue clusters distribution among *A. tritici*, *D. destructor* and *M. incognita* (D) Pairwise heat map indicating the cluster number overlapped in genome of each nematode species

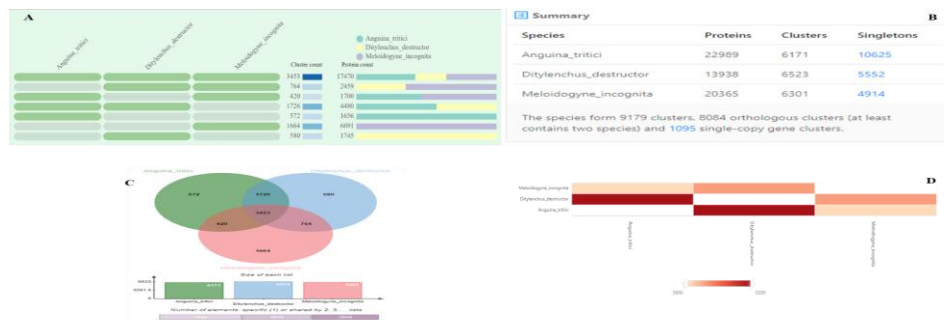


Fig.2 Details of Biological process, Molecular function and Cellular component

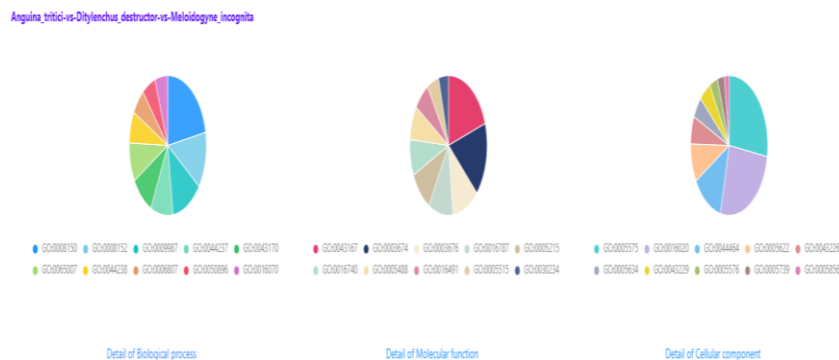
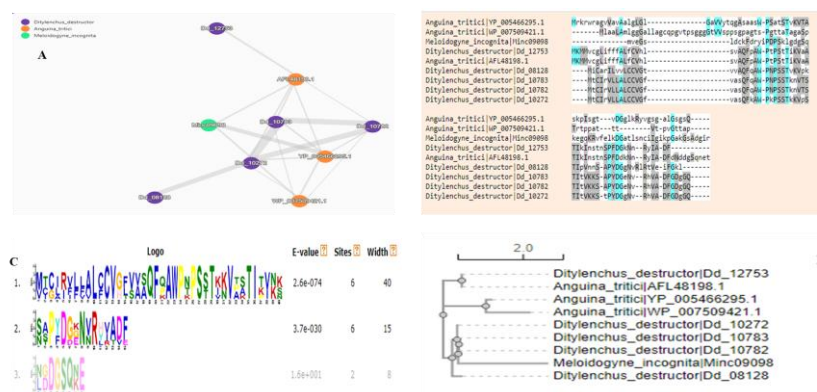


Fig.3 Annotation of cluster 63 showing Pectatelyase using different methods (A) protein network found with in cluster 63 among all three nematodes (B) Multiple sequence alignment of Pectatelyase protein sequence (C) Discovered Motifs in the protein sequence of cluster 263 with Pectatelyase function (D) Phylogenetic tree of Pectatelyase protein



Unique gene identified in *A. tritici* was largely abundant for scavenger receptor activity, nuclease activity and post-embryonic development (supp. link 4), while for *D. destructor* it was for pathogenesis and mRNA processing (supp link 5). The most unique clustered were found for *M. incognita* were largely enriched for ubiquitin-dependent protein catabolic process and zinc ion binding (supp link 6). Taking all these into account it implies that in all these three nematode species *A. tritici*, *D. destructor* and *M. incognita* genome have their own uniqueness in terms of genetic component. Therefore, we hypothesize that the multiple reference genome for genomic analysis will be valuable for comparative genomic study of nematodes.

Among all these genes we selected significant GO term involved in breakdown of pectin (an essential component of defence layer in plant root) with annotated accession cluster 63, ID GO:0045490. This gene was found to be shared among all these three-nematode species. In host parasite interaction effectors play an important role in breakdown cell wall which is consist of pectin and cellulose (Davis *et al.*, 2011). The presence of Pectatelyase in the *A. tritici*, *D. destructor* and *M. incognita* suggest that they might be playing similar functions. Conserved domain search for Pectatelyase protein common in all three species revealed to have interlinking cluster and similarity with each other (Fig. 3 A, B). In this study identified 6, 6, 2 sites for A.

tritici, *D. destructor* and *M. incognita* respectively with e-cutoff value 1.6 to 3.6 (Fig. 3C). In order to investigate the evolutionary relationship and homology of Pectatelyase gene found in *A. tritici*, *D. destructor* and *M. incognita* we performed multiple sequence alignment (Fig. 3 B) and reconstructed phylogenetic tree (Fig. 3D). the obtained result indicated that the Pectatelyase gene has been expanded in *A. tritici* and *D. destructor* while in *M. incognita* it is in low number as compared to other two nematode species (Fig. 3D). Additionally, the Pectatelyase of *A. tritici* found to be closely related to *D. destructor* than *M. incognita* (Figure 1A).

Occurrence table indicating shared orthologue groups patterns between *A. tritici*, *D. destructor* and *M. incognita*. Left pattern indicating the species of nematode involved in clusters, shared cluster count among them and protein count among the shared clusters (B) cluster count in genome of each nematode species. Singleton describing those genes having no orthologue; (C) Venn diagram showing the shared orthologue clusters distribution among *A. tritici*, *D. destructor* and *M. incognita* (D) Pairwise heat map indicating the cluster number overlapped in genome of each nematode species

In conclusion the genome sequencing effort for nematodes have generated the huge amount of genomic dataset. Comparative genomics between *A. tritici*, *D. destructor* and *M. incognita* revealed various numerical variation in genetic contents, conservation of developmental related genes and many unique genes specific to all three nematode species exclusively.

Supporting link

1. Result
page:<https://orthovenn2.bioinfotoolkits.net>

/task/result/c5f9a05ef0e1341cab68133f775d6c2d

2. <https://orthovenn2.bioinfotoolkits.net/overlap/c5f9a05ef0e1341cab68133f775d6c2d/jvnnOverlap/ortho?bin=QW5ndWluYV90cml0aWNpLXZzLURpdHlsZW5jaHVzX2Rlc3RydWN0b3ltdnMtTWVsb2lk2d5bmVfaW5jb2duaXRh&sp=QW5ndWluYV90cml0aWNpLERpdHlsZW5jaHVzX2Rlc3RydWN0b3ltdnMtTWVsb2lk2d5bmVfaW5jb2duaXRh>
3. <https://orthovenn2.bioinfotoolkits.net/task/result/c5f9a05ef0e1341cab68133f775d6c2d>
4. <https://orthovenn2.bioinfotoolkits.net/overlap/c5f9a05ef0e1341cab68133f775d6c2d/jvnnOverlap/ortho?bin=QW5ndWluYV90cml0aWNpLXZzLURpdHlsZW5jaHVzX2Rlc3RydWN0b3ltdnMtTWVsb2lk2d5bmVfaW5jb2duaXRh>

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