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# *In silico* analysis of *FLP 18* gene of *Anguina tritici* and phylogenetic analysis with other nematodes

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#### Abstract

 $flp \ 18$  is a neuropeptide coding gene of FMR Famide-like peptides family having important role in behavioural regulation such as locomotory, feeding and reproduction of nematodes. Genome annotation of the newly sequenced genome of seed gall nematode (*Anguina tritici*) has revealed the presence of flp18 in their genome.  $flp \ 18$  gene has been proven as effective target for management of *Meloidogyne incognita*. In view of importance of this gene here we analyse the significance of  $flp \ 18$  gene in *A. tritici* in different nematode species through the *In silico* approach. Homologus nucleotide sequences in other nematode species for the gene  $flp \ 18$  were obtained on performing nucleotide BLAST in WormBase Parasite database. Multiple sequence alignment, identity and phylogenetic analysis were performed on the retrived homologus sequences using CLUSTAL W programme. The BLAST result revealed the presence of  $flp \ 18$  hits in different groups of plant parasitic nematodes with best hit (76) to *Ditylenchus dipsaci*. Based on the finding it has been suggested that the  $flp \ 18$  of *A. tritici* is conserved with identical homologs, and might have functional similarity among different nematodes species.

Keywords: flp 18, seed gall nematode, Anguina tritici, In silico, phylogenetic analysis

#### Introduction

FMRF amide like peptides (FLPs) are the important genetic component of nematode nervous system and largest neuropeptide coding gene family conserved across the phylum Nematoda <sup>[1]</sup>. More than 70 FLPs which have been mapped extensively in *Caenorhabditis elegans* have revealed their expression in almost 302 neuronal networks <sup>[2]</sup>. FLPS have been known to regulate vivid biological functions including muscular contractions, neuromodulation, egg laying, and feeding behaviours of nematodes. Among all the FLPs, particularly the *flp 18* gene is crucial in nematodes which is responsible for synthesis of neuropeptide ligands of NPR-1 gene of GPCR (G Protein Couple Receptor) having role in foraging and aggregation of nematodes <sup>[3, 4, 5, 6, 7]</sup>. Availability of genomic data with annotated genetic information have revealed the distribution profile of *flp 18* in various nematode groups including Ascaris caninum, Ancyclostoma ceylanicum, A. suum, Bursaphelenchus xylophilus, Caenorhabditis elegans, D. immitis, Globodera pallida, G. rostochiensis, Haemonchus concortus, Loa loa, M. chitwoodi, M. hapla, M. incognita, M. javanica, N. americanus, Necator brasiliensis, O. ochengi, O. ostertagi, O. volvulus, Pristionchus pacificus, Strongylus ratti, S. stercoralis, Trichinella muris, T. spiralis, W. bancrofti and recently in Ditylenchus destructor <sup>[2, 8]</sup>, flp 18 gene has been playing a significant role in regulating key biological process of nematodes and is a potential gene target in plant parasitic nematodes (PPNs) management through RNAi <sup>[9]</sup>. However, considering the diversity of nematodes there is still lack of genomic information about *flp* 18 gene in comparison to nematode distribution. In contrast to the free-living nematodes, and animal and human parasitic nematode, among PPNs the diversity of *flp 18* are lesser known. Our genome sequencing effort of seed gall nematode (Anguina tritici) have confirmed the hits of *flp 18* gene complement which have not been reported so far from this species of plant parasite. This study expands our knowledge about presence of *flp 18* in Anguina tritici and diversity of its homologues, physicochemical properties and phylogenetic relationship of *flp 18* gene in various nematodes.

#### Material and Methods 1. Mining for the homologus DNA sequence of gene *FLP* 18

The nucleotide sequences of annotated *flp 18* gene (Figure 1) were obtained from the de-novo sequencing project of *Anguina tritici* (data unpublished in public domain).

Nucleotide sequence were translated into protein using EMBOSS Transeq <sup>[10]</sup>. *flp 18* sequences were subjected to BLAST using WormBase Parasite database <sup>[11]</sup>. Nematode species having identical homologs were retrieved and used for multiple sequence alignment (MSA).

Query_1:		GCCTTGATGGAGCCCAGTTTGGTGCAGAACAGTGGGAATGCTAAGTACGGCTCTTTCGAT	Query_1:	743
	1			60
Scaffold_1118:19	95220	GCCTTGATGGAGTCCACTTTGATGCAAAATGGTGCTAATGCTAAGTACGGCTCTTTTGAT	Scaffold_1118:1	95161
Query_1:	744	GTGGTGGCTCCGGTTT	Query_1:	759
	61	1111111111111111		76
Scaffold_1118:19	95160	GTGGTGGCTCCGGTTT	Scaffold_1118:1	95145

Fig 1: Alignment of FLP 18 nucleotide with Anguina tritici as query and Ditylenchus dipsaci as subject

### 2. Multiple sequence alignment and determination of identity

The similarity and percent identity among the retrieved sequences of *flp 18* were determined by using DNA database of WormBase BLAST. MSA were performed using CLUSTAL 2.1 Genome Net programme and function "build" of ETE3 v3.3.1 <sup>[12]</sup>.

#### 3. Phylogenetic analysis of *flp* 18

To deduce the phylogenetic relationship the phylogenetic tree was reconstructed using the MSA file of nucleotide sequences retrieved from CLUSTAL 2.1. The tree was inferred and evaluated using the MEGA X <sup>[13]</sup> with bootstrap value of 1000 replications and other default parameters.

#### 4. Prediction of physicochemical properties of *flp* 18

DNA sequence of *flp 18* was translated into protein using ExPASy Translate SIB resource programme <sup>[14]</sup>. Physicochemical properties including amino acid, atomic composition, isoelectric points, instability index etc. were predicted using ProtParam tool <sup>[15]</sup>.

#### **Results and Discussion**

The genome sequence analysis of nematodes has revealed large number of genes coding for proteins involved in key biological function of nematodes. Among all the biological function neurotransmission is highly significant due to its role in assimilation and interpretation of information from surroundings and enables nematodes to respond according to the stimuli. The process of synaptic transmission is carried out by vast range of neuropeptide coding genes. The FLPs genes are one such family of neuropeptide coding genes which are crucial player in various functions of nematode including feeding, reproduction and movement <sup>[2]</sup>. In the genomic analysis of A. tritici we have predicted presence of flp 18 which have been known in other nematodes including free living (C. elegans), parasite of vertebrate (Ascaris) and plant parasites (M. incognita, D. dipsaci) <sup>[2, 8]</sup>. To develop an understanding for the role of flp 18 gene in A. tritici we predicted its physicochemical properties and functionality in relationship with other nematodes. The total length of nucleotide sequence and translated amino acid sequence of *flp* 18 gene obtained from A. tritici presented a variation in length. The total length of *flp 18* nucleotide sequence was 1020 bp and amino acid were observed to be 325 (Table 1). BLASTN search tool of WormBase Parasite database for flp18 presented best similarity with D. dipsaci with alignment length of 76, alignment score-40, E value cut-off 2.0E<sup>-11</sup> and percent identity of 88.2% (Fig 1). This highest possible

similarity in both species suggests similar evolution history and differentiation of *flp-18* among both the species. BLASTN search revealed hits of *flp 18* within other nematodes including Meloidogyne spp., Plectus sambesii, Onchocerca flexuosa, Syphacia muris etc. (Table 2). Additionally, the query coverage for BLASTN were found highest (76) for D. dipsaci and lowest (21) for Strongylus vulgaris (Table 2). Homology of flp 18 were inferred using Multiple sequence alignment (MSA) for 13 retrieved biological sequences of different species through CLUSTAL programme. Phylogenetic analysis conducted to trace the origin of *flp 18* and their relation with other nematode species showed that in due course of evolution the *flp* 18 gene originated from common ancestors and diverged into two major branches. The *flp* 18 gene in A. tritici have been hypothecated to be more closely related to D. dipsaci (as they fall in same clade and having common divergence event and descendance) in comparison to other nematodes. there was less relatedness among other plant parasitic nematodes including M. incognita, M. floridiensis, M. enterolobii, M. javanica and M. arenaria. Similarily, PPNs like M. incognita, M. floridiensis, and M. javanica, M. arenaria were closely related to each other with respect to *flp 18* gene based on the evolutionary history (Fig 2). The *flp 18* protein analysis revealed various physicochemical properties (Table 3). The number of amino acids were translated 325 from nucleotide sequences and molecular weight of *flp 18* protein predicted to weigh 36770.94 kDa. The isoelectric point (pI) of *flp 18* were 10.22, the Total number of negatively charged residues (Asp + Glu) are lower than the total number of positively charged residues (Arg + Lys) suggests their role in intercellular <sup>[16]</sup>. The estimated half-life of *flp 18* protein revealed 1.1 hours which means the protein is classified as unstable on stability index <sup>[17]</sup>. *flp* 18 gene is a key gene having significant role in neuro transmission. C. elegans phenotype in RNAi mutant line of *flp 18* showed various disrupted functions including decreased response to odour, increased reversal post starvation enhanced response to dauer formation. a significant functional role in suppression of overexcitation in locomotory circuit was observed <sup>[18, 19, 20]</sup>. *flp 18* has been known to play a key role in chemoreception (host finding by PPNs). RNAi knockdown study of flp 18 in M. incognita decreased chemotaxis to tomato roots and reduced the infectivity by J2s. The root exudates of Chrysanthemum were found to reduce the expression of Mi-flp-18 hence having an effect on rate of infection of nematode in host plant <sup>[21]</sup>. Since Anguina tritici is also a PPN feeding on various part of the host plant from germinating stage (plumule) to tillering (leaves) and on the flowering of the plant seeds), it requires robust mechanism of neurotransmission and the presence of homologus gene *flp 18* having close relation with other nematodes might give

insights into same functional properties and similar role in chemoreception and movement of the nematode.

#### Table 1: Nucleotide and translated amino acid sequences of flp 18

#### # DNA sequence

#### # 5'3' Frame amino acid sequence

RCLLNILSYIMAKNCGQGLLTHQSGHCSVVEVGCPIWEPQAILGWTSRGFNHLKDCGKKLVFHQMSNKKSISKMEFVARLHPPSGRCR VLNQFTILEFQPFTCNCEPPSGSLCLSASNNALKQRVTRKLQPRMVGRCKPTVVNKGGREPTNKASKDGNPPPSNRPDGLAKSRPMMG GCCWPLESDPATGTMRRTAQRRTWWRGTNRPLPQPPRPWSPVWCRTVGMLSTALSMWWLRFIHLFPSLVLRLDWLEKGQKSRRGMA CFLLGSACVFVTLFAKISVIPPVASKQQLPESFITGKYNSSLYFQPRSYENHRLDRNP

Genome	Genomic Location		Score	E-val	%ID
Ditylenchus dipsaci	Scaffold_1118:195145-195220	76	40	2.0E-11	88.2
Meloidogyne arenaria	M.Arenaria_Scaff525:34856-34886	31	23	0.28	93.5
Meloidogyne floridensis	scf7180000424632:8383-8413	31	23	0.28	93.5
Meloidogyne javanica	scf7180000424632:8383-8413	31	23	0.28	93.5
Meloidogyne enterolobii	scaffold26088_cov370:1071-1101	31	23	0.28	93.5
Meloidogyne incognita	FXSY01000192.1:10905-10935	31	23	0.28	93.5
Plectus sambesii	PSAMB.scaffold14934size1735:224-249	26	22	1.1	96.2
Onchocerca flexuosa	O_flexuosa-1.0_Cont1663:171459-171479	21	21	4.4	100.0
Syphacia muris	SMUV_contig0000193:6417-6437	21	21	4.4	100.0
Pristionchus entomophagus	scaffold710:48350-48374	25	21	4.4	96.0
Teladorsagia circumcincta	TELCIRDFT_Contig2292:32490-32510	21	21	4.4	100.0
Strongylus vulgaris	SVUK_contig0031032:980-1000	21	21	4.4	100.0

#### Table 2: Diversity of FLP 18 obtained in nematode species

Table 3: Physicochemical p	properties of FLP 18
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Number of amino acids:	325	
Molecular weight:	36770.94	
Theoretical pI:	10.22	
	Ala (A) 15	4.6%
	Arg (R) 28	8.6%
	Asn (N) 16	4.9%
	Asp (D) 6 1.8%	
	Cys (C) 15	4.6%
	Gln (Q) 14	4.3%
	Glu (E) 11	3.4%
	Gly (G) 23	7.1%
	His (H) 7 2.2%	
Amine said composition.	Ile (I) 10 3.1%	
Amino acid composition:	Leu (L) 32	9.8%
	Lys (K) 19	5.8%
	Met (M) 11	3.4%
	Phe (F) 14	4.3%
	Pro (P) 29	8.9%
	Ser (S) 28	8.6%
	Thr (T) 16	4.9%
	Trp (W) 10	3.1%
	Tyr (Y) 4 1.2%	
	Val (V) 17	5.2%
	Pyl (O) 0 0.0%	
	Sec (U) 0 0.0%	
Total number of negatively shored residues (A ) Ol)	17	
Total number of negatively charged residues (Asp + Glu)		

Total number of positively charged residues (Arg + Lys)	47
	Carbon C 1630
	Hydrogen H 2580
Atomic composition	Nitrogen N 482
	Oxygen O 438
	Sulfur S 26
	Formula: C1630H2580N482O438S26
total number of atoms	5156
The estimated half-life	1.1 hours (mammalian reticulocytes, in vitro).
Tu - 4-1:114-1:	The instability index (II) is computed to be 50.63
Instability inde	This classifies the protein as unstable
Aliphatic index	70.18

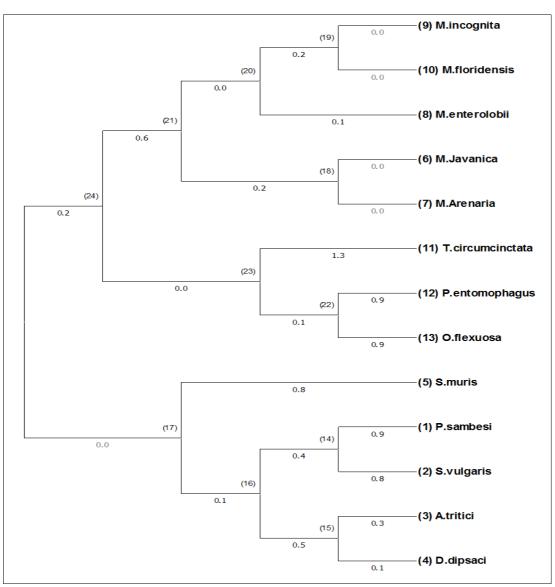


Fig 2: Phylogenetic tree of FLP 18 showing relationship with other nematode species

#### Conclusion

The *flp* 18 gene is an important genetic component of nematodes nervous system having a key role of chemosensation in different nematodes. Genomic sequencing and analysis have revealed their presence in different nematode group and in few cases their role has been confirmed through generation of RNAi mutant phenotype. In genome sequencing and annotation study of seed gall nematodes (*Anguina tritici*) have predicted the presence of *flp-18*. The in-depth in-silico analysis revealed they may have evolutionary history close to the *Ditylenchus dipsaci* and other nematodes. With the present study we hypothecate that the *flp* 18 protein might be playing similar role in *A. tritici* as

it does in other group of nematodes and in future it could be potential target for RNAi to understand functional genomics in *A. tritici* and their management through the novel approaches.

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