



E-ISSN: 2320-7078

P-ISSN: 2349-6800

www.entomoljournal.com

JEZS 2020; 8(5): 2433-2437

© 2020 JEZS

Received: 18-07-2020

Accepted: 23-08-2020

Ashish Kumar Singh

1. Division of Nematology,
ICAR-Indian Agricultural
Research Institute, Pusa
Campus, New Delhi, India

2. Division of Crop protection,
ICAR-Vivekananda Parvatiya
Krishi Anusandhan Sansthan,
Almora, Uttarakhand, India

Ila Joshi

ICAR-National Institute for
Plant Biotechnology, Pusa
Campus, New Delhi, India

Kuldeep Kumar

ICAR-National Institute for
Plant Biotechnology, Pusa
Campus, New Delhi, India

Pradeep Kumar Jain

ICAR-National Institute for
Plant Biotechnology, Pusa
Campus, New Delhi, India

Anil Sirohi

Division of Nematology, ICAR-
Indian Agricultural Research
Institute, Pusa Campus, New
Delhi, India

Corresponding Author:**Ashish Kumar Singh**

1. Division of Nematology,
ICAR-Indian Agricultural
Research Institute, Pusa
Campus, New Delhi, India
2. Division of Crop protection,
ICAR-Vivekananda Parvatiya
Krishi Anusandhan Sansthan,
Almora, Uttarakhand, India

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

Ashish Kumar Singh, Ila Joshi, Kuldeep Kumar, Pradeep Kumar Jain, and Anil Sirohi

DOI: <https://doi.org/10.22271/j.ento.2020.v8.i5ag.7838>

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 *flp 18* 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. Homologous 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 retrieved homologous 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 [1]. More than 70 FLPs which have been mapped extensively in *Caenorhabditis elegans* have revealed their expression in almost 302 neuronal networks [2]. 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 [3, 4, 5, 6, 7]. Availability of genomic data with annotated genetic information have revealed the distribution profile of *flp 18* in various nematode groups including *Ascaris caninum*, *Ancylostoma ceylanicum*, *A. suum*, *Bursaphelenchus xylophilus*, *Caenorhabditis elegans*, *D. immitis*, *Globodera pallida*, *G. rostochiensis*, *Haemonchus contortus*, *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* [2, 8]. *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 [9]. 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.

neurotransmission and the presence of homologous 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
<p>Agatgctctttgtaaaacatacttctactaaataatggcctaaatgtgggcaaggacttttgacgcaccagtcaggacactgtagtgggagggttggttcccatctgggaaacctcaagcaatgggctgga ccagtagaggggtcaaccattgaaagactgtggaataaagttatgtttccaccaatagatgtcaataaaagtctattagcaagatggagtcgtagctcgttaaacttaacactgaccgcacataaggaagatgc cgggtcctctaaaacaaftcaaatcctcgaafttcagccttctgaactgcaactgcgagccgcctctggcagcttagctcctagtcctcaaaacaatgcctgaaacaaagataagtgacgagaagctgcagc caagaatgtggggccgggtgcaaacatgaacttgagtggtcaacaaggaggaggagagcaacaacaaagctcaaaagacggcaatcctccccgtcaaatcggccagacgggttagccaaaagccggcc aatgtgaatggggggttggctggccactggagagttgagaccggccaccgggacatgagggagcagcacagcgtcgataaacctggggagggaacgaatcgccattaccacagccacctggcctt gatggagccagtttgggcaaacagtggaatgtaagtagcggcttttcgatgtgggtcgcggtttatacacctgttccatcctcagttttgagattggattggttagaagggggcagaagagctgatgaagg cggtaggggtgatagatggcgtgtttcttttaggttcagcatgtgtgtttgactttatttgcacaaaatacagttattccacttaggtggcctcaaacagcaattacctgagagctttataactggaaagtaaatcctc ctttatttccagcctagaaactgagaaacatagattagatagaaacccctg</p>
5'3' Frame amino acid sequence
<p>RCLLNILSYIMAKNCGQLLTHQSGHCSVVEVGCPIWEPQAILGWTSRGNHLKDCGKLVFHHQMSNKKSSISKMEFVARLHPPSGRCR VLNQFTILEFQPFTCNCEPPSGLSASNNALKQRVTRKLQPRMVGRCKPTVVNKGGREPTNKASKDGNPPSPNRPDGLAKSRPMMG GCCWPLES DPATGTMRRTAQRRTWWRGTNRPLPQPPRPSPVWCRTVGMSTALSMWWLRFIHLFPSLVLRDLWLEKGQKSRRGMA CFLGSA CVFVTLFAKISVIPVASKQQLPESFITGKYNSSLYFQPRSYENHRLDRNP</p>

Table 2: Diversity of FLP 18 obtained in nematode species

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

Table 3: Physicochemical properties of *FLP 18*

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

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

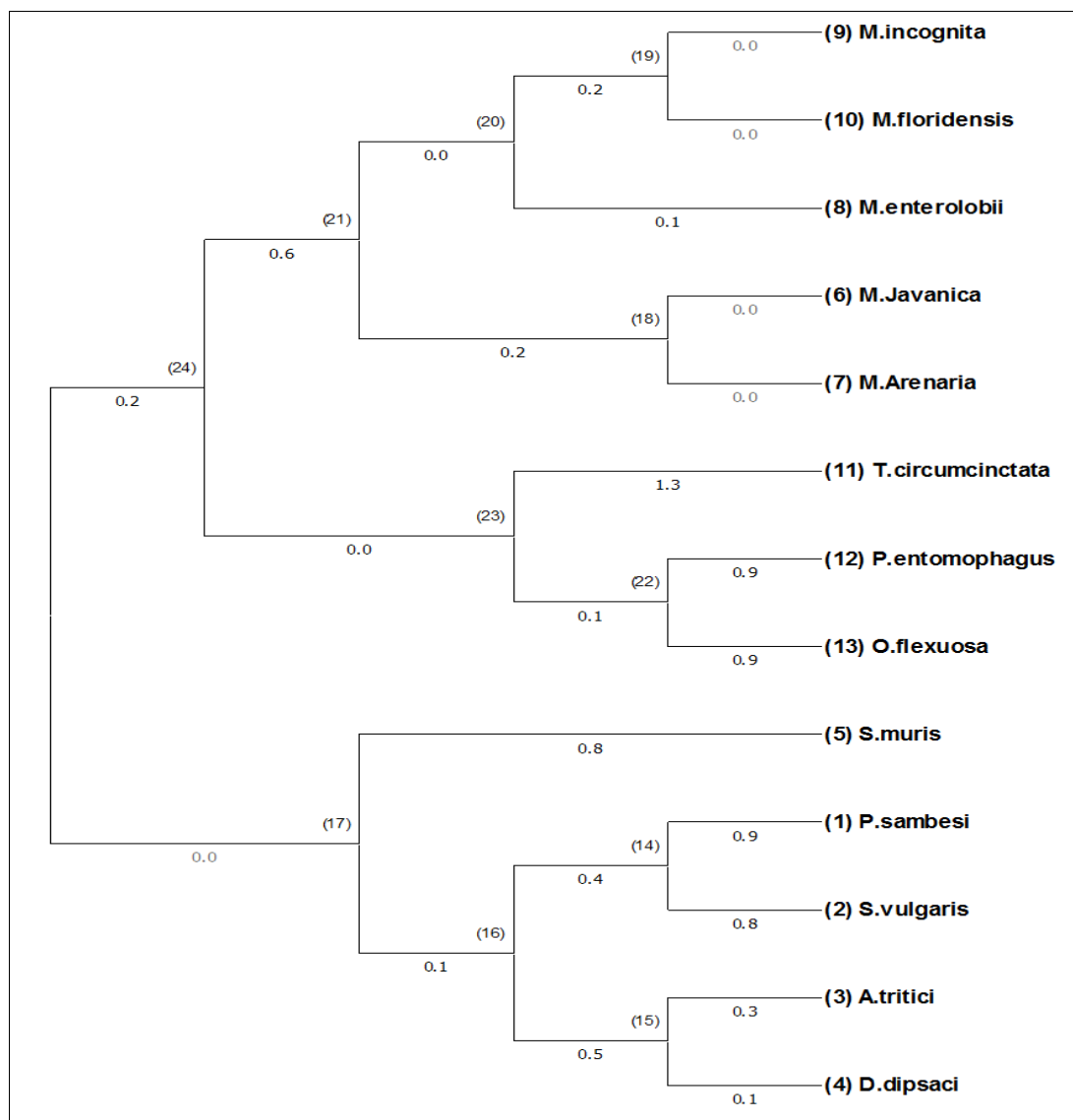


Fig 2: Phylogenetic tree of FLP 18 showing relationship with other nematode species

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 hypothesize 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.

Acknowledgements: The Authors are thankful to ICAR-NIPB and ICAR-IARI for providing the necessary computational resource for this work

References

1. McVeigh P, Leech S, Mair GR, Marks NJ, Geary TG, Maule AG. Analysis of FMRamide-like peptide (FLP) diversity in phylum Nematoda. *Int. J Parasitol*

- 2005;35(10):1043-60.
2. Peymen K, Wattey J, Frooninckx L, Schoofs L, Beets I. The FMR Famide-Like Peptide Family in Nematodes. *Frontiers in Endocrinology* 2014;5:90.
 3. Cowden C, Stretton AO, Davis RE AF1, a sequenced bioactive neuropeptide isolated from the nematode *Ascaris suum*. *Neuron* 1989;2(5):1465-73.
 4. Cowden C, Sithigorngul P, Brackley P, Guastella J, Stretton AO. Localization and differential expression of FMRFamide-like immunoreactivity in the nematode *Ascaris suum*. *J Comp Neurol* 1993;333(3):455-68.
 5. Li C, Nelson LS, Kim K, Nathoo A, Hart AC. Neuropeptide gene families in the nematode *Caenorhabditis elegans*. *Ann NY Acad Sci* 1999;897(6):239-52.
 6. Waggoner LE, Hardaker LA, Golik S, Schafer WR. Effect of a neuropeptide gene on behavioral states in *Caenorhabditis elegans* egg-laying. *Genetics* 2000;154(3):1181-92.
 7. Dockray GJ. The expanding family of FRFamide peptides and their effects on feeding behaviour. *Exp Physiol* 2004;89(3):229-35.
 8. Zheng J, Peng D, Chen L *et al.* The *Ditylenchus destructor* genome provides new insights into the evolution of plant parasitic nematodes. *Proc Biol Sci* 2016;283(1835):20160942.
 9. Papolu PK, Gantasala NP, Kamaraju D, Banakar P, Sreevathsa R, Rao U. Utility of host delivered RNAi of two FMRF amide like peptides, *flp14* and *flp-18*, for the management of root knot nematode. *Meloidogyne Incogn.* *PloS One* 2013;8(11),e80603.
 10. Kanz C, Aldebert P, Althorpe N *et al.*, The EMBL Nucleotide Sequence Database. *Nucleic Acids Res* 2005;33:D29-D33.
 11. Kevin L. Howe, Bruce J. Bolt, Myriam Shafie, Paul Kersey, and Matthew Berriman. WormBase ParaSite a comprehensive resource for helminth genomics *Molecular and Biochemical Parasitology* 2017;215:2-10.
 12. Huerta-Cepas J, Serra F, Bork P. ETE 3: Reconstruction, Analysis, and Visualization of Phylogenomic Data. *Mol Biol Evol* 2016;33(6):1635-1638.
 13. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 2018;35:1547-1549.
 14. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res* 2003;31:3784-3788.
 15. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD. Protein Identification and Analysis Tools on the ExPASy Server; (In) John M. Walker (ed): *The Proteomics Protocols Handbook*, Humana Press 2005,571-607
 16. Andrade MA, SI O'Donoghue, B Rost. Adaptation of protein surfaces to sub cellular location. *J Mol. Biol* 1998;276:517-525.
 17. Guruprasad K, Reddy BVB, Pandit MW. Correlation between stability of a protein and its dipeptide composition: A novel approach for predicting *in vivo* stability of a protein from its primary sequence. *Protein Eng. Des. Sel* 1990;4:155-161.
 18. Pierce SB, Costa M, Wisotzkey R, Devadhar S, Homburger SA, Buchman AR, *et al.* Regulation of DAF-2 receptor signaling by human insulin and *ins-1*, a member of the unusually large and diverse *C. elegans* insulin gene family. *Genes Dev* 2001;15:672-86.
 19. Nelson LS, Kim K, Memmott JE, Li C. FMRFamide-related gene family in the nematode, *Caenorhabditis elegans*. *Brain Res Mol Brain Res* 1998;58:103-11.
 20. Stawicki TM, Takayanagi-Kiya S, Zhou K, Jin Y. Neuropeptides function in a homeostatic manner to modulate excitation-inhibition imbalance in *C. elegans*. *PLoS Genet* 2013;9(5):e1003472.
 21. Dong L, Li X, Huang L, Gao Y, Zhong L, Zheng Y *et al.* Lauric acid in crown daisy root exudate potently regulates root-knot nematode chemotaxis and disrupts *Mi-flp-18* expression to block infection. *J Exp Bot* 2014;65:131-41.