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RKN Lethal DB: A database for the identification of Root Knot Nematode (*Meloidogyne spp.*) candidate lethal genes

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Abstract:
Root Knot nematode (RKN; *Meloidogyne spp.*) is one of the most devastating parasites that infect the roots of hundreds of plant species. RKN cannot live independently from their hosts and are the biggest contributors to the loss of the world's primary foods. RNAi gene silencing studies have demonstrated that there are fewer galls and galls are smaller when RNAi constructs targeted to silence certain RKN genes are expressed in plant roots. We conducted a comparative genomics analysis, comparing RKN genes of six species: *Meloidogyne Arenaria*, *Meloidogyne Chitwoodi*, *Meloidogyne Hapla*, *Meloidogyne Incognita*, *Meloidogyne Javanica*, and *Meloidogyne Paranaensis* to that of the free living nematode *Caenorhabditis elegans*, to identify candidate genes that will be lethal to RKN when silenced or mutated. Our analysis yielded a number of such candidate lethal genes in RKN, some of which have been tested and proven to be effective in soybean roots. A web based database was built to house and allow scientists to search the data. This database will be useful to scientists seeking to identify candidate genes as targets for gene silencing to confer resistance in plants to RKN.

Availability: The database can be accessed from: http: / / bioinformatics.towson.edu / RKN /

Keywords: RKN, *Meloidogyne*, web based database, RNAi, *C. elegans*

Background:
Root knot nematodes (RKN; *Meloidogyne Spp.*) are obligatory parasites that infect the roots of hundreds of plant species. The annual global agricultural losses of RKN have been estimated to be $157 billion **[1]**. The RKN is considered to be a major pathogen of vegetables throughout the globe. It impacts both the quantity and quality of crop yields **[2]**. The RKN has a wide host range; all major field crops, fruit trees, most vegetable crops, ornamental plants, and weeds are affected. Many of the traditional agricultural control practices (crop rotation, adding organic matter, removing diseased plants, and using resistant varieties) have not managed to control the parasite. And the use of chemicals has proven to be ineffective and hazardous. Therefore new approaches are needed. One would be to create transgenic plants using RNAi targeted to silence essential nematode genes **[3]**. We therefore set out to identify genes in RKN that would be lethal if silenced or mutated. We developed a pipeline and database to store our results. The genes were placed in a web based database for easy searching by the biological community.

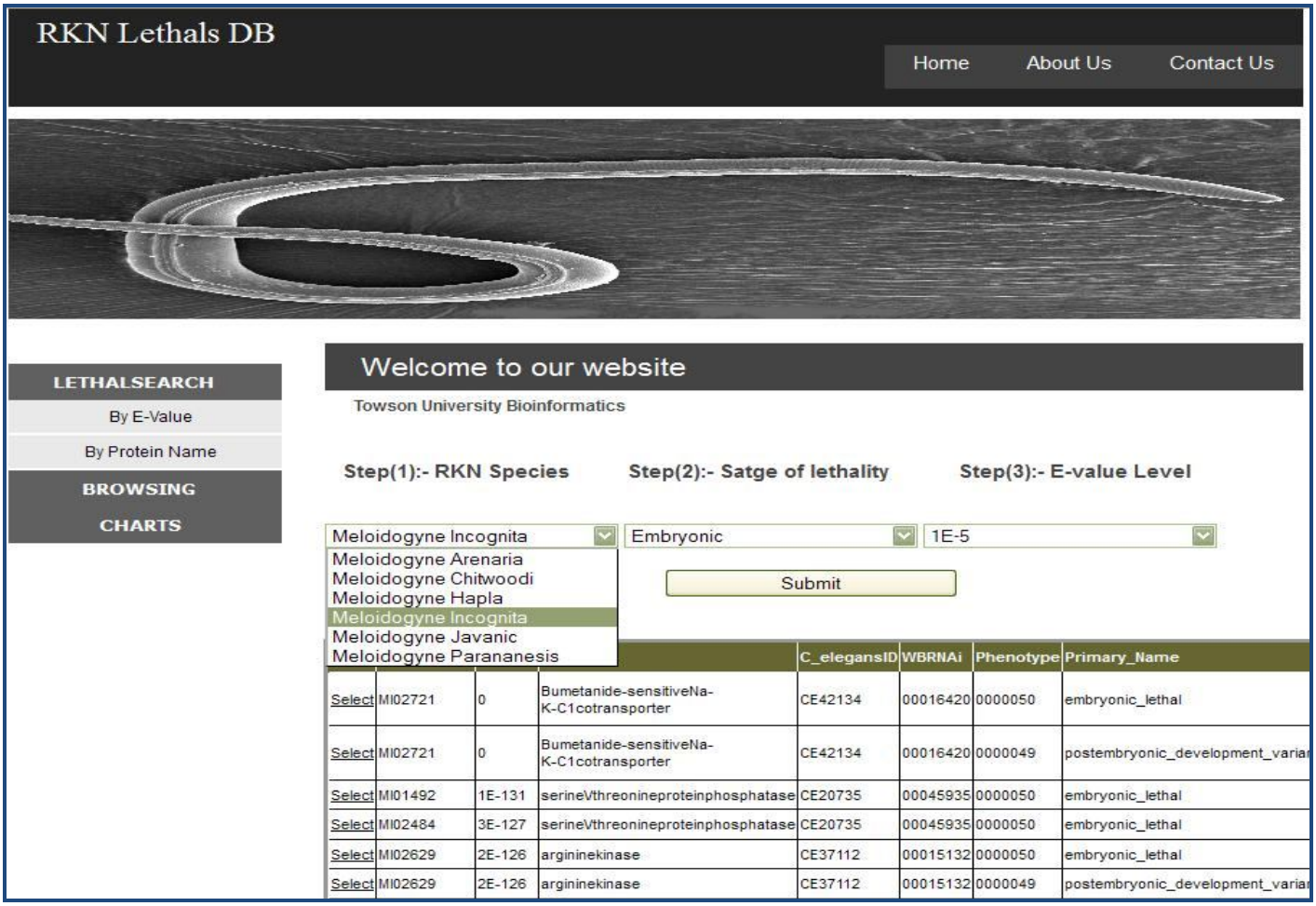

Figure 1: Snap shot of one of the search pages on the RKN lethal database site.

Methodology:

We conducted a comparative genomics study of all known EST sequences of RKN (from all 6 known species) with that of the well characterized and free living nematode *Caenorhabditis elegans*. We identified conserved genes that are candidates for being lethal at any stage of the RKN if mutated or silenced. We chose *C. elegans* because unlike RKN, it is well-characterized and there are numerous knock out and RNAi studies that have been done on it, making it a perfect species for comparison.

We downloaded all known EST and genomic sequences for *C. elegans* from Wormbase (www.wormbase.org; 356,773 EST sequences and 41,195 Genomic sequences in total). A unigene set was generated from these sequences by using the sequence assembly program Cap3 **[4]**. We also downloaded all known preassembled (unigene sets) ESTs for each one of the six species of the RKN from (http://www.nematode.net). Then we imported these sequences into our local SQLServer database. The RKN unigene sets were compared against the *C. elegans* sequences using the stand-alone blast software from NCBI (http://www.ncbi.nlm.nih.gov/). Perl scripts were written to parse the results of the blast searches. The parsed blast results were also stored in the database. SQL scripts were written to query the database to identify RKN genes with high similarity to *C. elegans* (e-value <= 1E-5). Those genes were then mapped against Wormbase to find out which of these genes resulted in an embryonic, larval, or adult lethal phenotype in *C.elegans* if mutated or silenced, following the procedure described in **[5]**. We developed a web based user interface and provided search capabilities for scientists in the field. The search for candidate lethals could be performed by using the species name, stage of lethality, and E-value level and\or by gene\protein name **(Fig 1)**. The user interface was developed using ASP.NET.

Utility to the biological community:

We identified 7 candidate lethal genes from *M.arenaria,* 2260 genes from *M.chitiwoodi,* 5611 genes from *M.hapla*, 5650 genes from *M.incognita*, 2932 genes from *M. javanica,* and 1052 from *M.paranaensis.* RNAi constructs against four genes in *M.incognita* have already been made and proven to be effective in soybean roots **[6]**. Overall these results show a promising solution for broadening resistance of plants against this plant-parasitic nematode.

Caveats:

As the genome sequence of *M.incognita* becomes better characterized, this study can be updated to identify further candidate lethal genes.

Future Development:

We hope to also incorporate gene expression experiments within our database to show the level of expression of the candidate lethal genes in different biological experiments and species.

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