

## Comparative analysis and structure elucidation of Syntaxin- A novel component tends to defense mechanism in plant proteomics.

Saurabh Shukla<sup>1</sup>, Prashant Srivastava<sup>1</sup>, Sanjay Kumar Choubey<sup>1</sup> and Gomase V.S.<sup>2</sup>

<sup>1</sup>Yeshwant College of Information Technology (Bioinformatics & Biotechnology), Parbhani, MS

<sup>2</sup>School of Technology, S.R.T.M. University, Sub-Centre, Latur, 413512, India

**Abstract**-Syntaxin is one of the plants housekeeping resistance protein, occurred in most of the plants. Plants resist microbial attack using elaborate nonself surveillance systems consisting of a repertoire of cell surface and intracellular immune sensors. Thus plants must have evolved defense mechanisms to terminate extracellular colonization attempts. The plant must, therefore, promote an active resistance mechanism to combat the extracellular infection. Resistance against bacteria is manifested and whether similar processes mediate basal, gene-for-gene, and salicylate-associated defense, however, so many resistant proteins are poorly understood. The influence of plasma membrane syntaxin is a component contributing to gene-for-gene resistance in *Nicotiana benthamiana*. Silencing the apparent orthologue of a syntaxin required for resistance to powdery mildew fungus, compromised resistance because syntaxins may play a role in secretion of proteins to the extracellular space, proteomic analysis of the apoplastic fluid. Syntaxin silenced plants were impaired in the accumulation of at least a subset of pathogenesis-related (PR) proteins in the cell wall. SYP132-dependent secretion is a component of multiple forms of defense against bacterial pathogens in plants. Thus, we are implementing the Phylogenetics or comparative approach towards the syntaxin sequences observed in multiple plant genomes.

### Introduction

Plant disease resistance is one of the challenging aspects in the system biology. Syntaxin is one of the conserved component in many plants. As per the observation in genetic analyses, syntaxin SYP121 in *Arabidopsis* is required for penetration resistance. SYP121 is probably necessary for vesicle trafficking leading to formation of papillae, which are local cell wall appositions functioning as barriers against fungal penetration. The closely related SYP122 is not required for penetration resistance. Other defense mechanisms are controlled by different signaling pathways, which are activated upon pathogen attack. The signaling compounds salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) each define such pathways. In addition, plant cells can undergo the "hypersensitive response" (HR), a program cell death (PCD) reaction, that confer resistance to biographic pathogens. Examined the involvement of SYP121 and SYP122 in these four signaling pathways, and found that the syntaxins act as negative regulators of all four. While SYP121 is the primary regulatory protein, SYP122 is partially able to take over it role. The release of this negative regulation in the syp121 syp122 double mutant results in

strong PCD-mediated resistance to an otherwise virulent powdery mildew fungus.

### Materials and methods

#### Retrieval of existing syntaxin protein sequences from 17 different plants.

- Select the protein from protein data base for syntaxin
- We find out the 17 sequences for syntaxin protein from 17 different plants Species.

1) syntaxin [Machiloides banksi] gi|262304039|gb|ACY44612.1| KKHSAILSAPQSDEKTKQELEDLMADIKKN ANKVRRAKVKVIEQNEEQEQTNKSSADLRI RKTQHLSLSRKFVEVMTEYNRTQTDYRER CKGRIQRQLEITGRITTTNEELEEMLEQGN PSVFTEGIVMDTTQAKQTLADIEARHADIK LETSIKEL

2) syntaxin [Prokopenia wheeleri] gi|262304059|gb|ACY44622.1| KKHSAILSAPQTDEKVKQELDDMMADIKKT ASRVRTKLKVMQNEQMEQTNKSSADFR IRKTQHSMLSQKFVEVMTDYNKTQTDYRE RCKARIQRQLEITGKVTNTNEELEEMLESGN PAIFTQGIIMDTQAAKQTLADIEARHADIMK LESSIREL

- 3) syntaxin [*Scolopendra polymorpha*]  
gi|262304067|gb|ACY44626.1  
KKHSAILSAPQTDEKIKQELDDLTDDIKKRA  
NKVRAKLVKIEQSIEQEELELNIEKDEPLNMS  
DAELRIRKTHFTLTKRFVEIMTEYNRTQV  
DHRDRCKGRIQRQLEIAGKETTDEELEDML  
QSGNPAIFTQDIETDTQQAQKTLADIEARH  
EDIKLENSIREL
- 4) syntaxin [*Skogsbergia lernerii*]  
gi|262304065|gb|ACY44625.1|  
MKHSAILSAPQADEKDKQELEDLMTDIKK  
MANRVRAKLVKIEQNIQEEQTNKTSADLR  
IRKTQQSTLSRKRFVEVMTEYNRTQTDYRE  
RCKGRIQRQLEITGRTTTTNDELEDMLEKG  
NPSVFTQGIIMETQQAQKTLADIEARHADI  
MKLEKSIQEL
- 5) syntaxin [*Stenochrus portoricensis*]  
gi|262304069|gb|ACY44627.1|  
KKHSAILSAPQTDEKVKQELEDLMADIKKT  
ANRVRAKLVKMEQNIQLEQTSMMMSADFR  
IRKTQHSMLSQKFVEVMTEYNRTQTDYRE  
RCKARIQRQLEITGKVTTNDELEEMLESGN  
PAIFTQGIIMETQQAQKTLADIEARHADIKL  
ETSI
- 6) syntaxin [*Streptocephalus seali*]  
gi|262304073|gb|ACY44629.1|  
MKHSSILSAPQTDEKVKQELEDLMADIKKT  
ANKVRRKLDIEQNIQEEQKNKPNADFRI  
RKTQHSTLSRKRFVEVMTEYNKTQTDYRER  
CKARILRQLEITGRQTTDQEELEEMLEQGNP  
AVFTQGIIMETQQARQTLADIEARHADIKL  
ENSIREL
- 7) syntaxin [*Tomocerus Tom2*']  
gi|262304071|gb|ACY44628.1|KKHSAILS  
PTTDEKMKQELEDLMAGIKKTANQVRSKL  
KVIEQNIQEEHTNKSSADLRIRKTHSTL  
SRKFVEVMTEYNRTQTDYRERCKGRIQR  
QLEITGRTTTTDEELEMIEQGNPAVFTQGI  
METQQARQTLADIEARHADIKLENSIREL
- 8) syntaxin [*Nicoletia meinerti*]  
gi|262304043|gb|ACY44614.1  
KKHSSILSAPQTDEKVKQELEDLMADIKKT  
ANKVRAKLVKIEQNIQEEHTNKSSADLRIR  
KTQHSTLSRKRFVEVMTEYNRTQTDYRERC  
KGRIQRQLEITGRTTTTNDELEEMLEQGNP  
AVFTQGIIMETQQAQKTLADIEARHADIKL  
ENSIREL
- 9) syntaxin [*Nicotiana tobacum*]  
gi|218744540|dbj|BAH03479.1|  
MSFDLEAGRLLGPRRGYLNKQDPTQA  
MVSDLPINTAVSTFQRLVNTLGTDPKDP  
REKLHKTRVHIGQLVKDTSALKKQASETD  
HRIEVSASKITDAKLAKDFQAVLKEFQKA  
QRLAAERETAYTPFVPAVLPSSYTASEID  
VSTESPEQRAFLVESRRQEVLLLDNEIAF  
NEAIIEREQGIQEIQQQIGEVNEIFKDLAVL
- VHEQGAMIDDIGSNVENAHAATAQGRSQL  
AKAAKTQRSNSSLTCLLLVIFGIVLLIVVVL  
AA
- 10) Santaxin [*Peripatoides novaezealandiae*]  
gi|262304053|gb|ACY44619.1  
KKHSAILSAPQPDEKVKEELEELMADIKKT  
ANKVRGKLTIEQNIQEEHTNKSSADLRIR  
RKTQHSTLSRKRFVEVMTEYNRTQTDYRER  
CKGRIQRQLEITGRTTTTNEELEDMLESGNP  
AIFTQGIIMETQQARQTLADIEARHNDIIKLE  
TSIREL
- 11) syntaxin [*Periplaneta americana*]  
gi|262304045|gb|ACY44615.1|  
KKHSAILSAPQTDEKVKQELEDLMADIKKT  
ANKVRAKLVKIEQAVEQEEQTNKSSADLRIR  
RKTQHSTLSRKRFVEVMTEYNRTQTDYRER  
CKGRIQRQLEITGRTTTTNEELEMLEQGN  
PAVFTQGIIMETQQAQKTLADIEARHADIK  
LENSIREL
- 12) syntaxin [*Peripatus sp. 'Pep'* ]  
gi|262304047|gb|ACY44616.1  
KKHSAILSAPQPDEKVKEELEELMADIKKT  
ANKVRGKLSIEQNIQEEHTNKSSADLRIR  
RKTQHSTLSRKRFVEVMTEYNRTQTDYRER  
CKGRIQRQLEITGRTTTTNEELEDMLESGNP  
AIFTQGIIMETQQAQKTLADIEARHNDIIKLE  
NSIREL
- 13) syntaxin [*Polyxenus fasciculatus*]  
gi|262304055|gb|ACY44620.1  
KKHSAILSAPQTDEKVKQELEDLMADIKKT  
ANKVRAKLVKIEQNIETEEHTNKSSADLRIR  
KTQHSTLSRKRFVEVMTEYNRTQTDYRERC  
KGRIQRQLEITGRTTTTNEELEDMLESGNPA  
VFTQGIIMETQQAQKTLADIEARHADIKLE  
NSIREL
- 14) ) syntaxin [*Phrynus marginemaculatus*]  
gi|262304051|gb|ACY44618.1  
KKHSAILSAPQTDEKVKQELEDLMADIKKT  
ANRVRAKLVKMEQNIQLEQTSMMMSADFR  
IRKTQHSMLSQKFVEVMTEYNRTQTDYRE  
RCKARIQRQLEITGKVTTNDELEEMLESGN  
PAIFTQGIIMETQQAQKTLADIEARHADIKL  
ENSIREL
- 15) syntaxin [*Scutigera sp. 'Scu3'*]  
gi|262304063|gb|ACY44624.1  
KKHSSILSAPQTDEKIKQELEDLMADIKKT  
NRARAKLKAIEQNIQEEHTNKSSADLRIR  
KTQHSTLSRKRFVEVMTEYNRTQTDYRERC  
KGRIQRQLEITGKSTTDDEIEDMLESGNLQ  
VFTGGIVMDSAQAKQTLADIEARHNDIIKLE  
TSIREL
- 16) syntaxin [*Neogonodactylus oerstedii* ]  
gi|262304041|gb|ACY44613.1|  
KKHSEILSAPQTDDKMKQELEDMMMTDIKK  
TANRVRAKLVKIEQSIETEEQQSKTSADLRIR  
KKTQHSTLSRKRFVEVMTEYNRTQTDYRER

CQRQLEITGRNHTSDEVEDMLEQGNPAVF  
TQGIIMETQQAQKQTLADIEARHADIMKLEN  
SIREL.

17) syntaxin [Pedetontus saltator]  
gi|262304057|gb|ACY44621.1|  
KKHSAILSAPQSDEKTKQELEDLMADIKKN  
ANKVRAKVKVIEQNIQEEQTNKSSADLRI  
RKTQHLSLSRKFVEVMTEYNRTQTDYRER  
CKGRIQRQLEITGRTTTTNEELEEMLEQGN  
PSVFTEGIVMDTNQAKQTLADIEARHADIIK  
LETSIKEL

For the Phylogenetic analysis and protein secondary structure prediction we need to collect the sequential data in a proper format. For this we used all sequences in fasta formats.

#### Phylogenetic analysis of syntaxin protein

Open the Clustal X window

Append the sequences in fasta format

Do complete alignment.

Save the file as .dnd syntaxin

Open the tree view software

Import the file dnd syntaxin and print the Phylogenetic tree.

#### Conserved sequences by using Gene doc

Open the window of Gene doc.

Import the sequences in fasta format.

Done.

Give the conserved sequences.

Save the file as syntaxin

#### Root distances and pair distances by Using phylodraw

Open phylip window

Import the gene doc file and done

Root distances and pair distances  
Calculated in the pair distances (distances  
between plant shows same ancestors.

#### Secondary structure by using Sopma

Open the Sopma software on line

Pest the sequences in quire window

And OK

Give all information about the secondary structure

#### 3-D structure by using Geno3d

Open the Geno3d home page

Paste the sequence in query window

Click on submit button

Give the all information about tertiary structure

#### Results

The molecular mechanisms of receptor-mediated non-self recognition and subsequent intracellular signaling pathways have received much attention in the past.

Less is known about the cellular mechanisms that contribute to the execution of immune responses. Plant disease resistance is the result of the collective activity of several separate defense mechanisms. In genetic analyses, syntaxin SYP121 in Arabidopsis is required for penetration resistance. SYP121 is probably necessary for vesicle trafficking leading to formation of papillae, which are local cell wall appositions functioning as barriers against fungal penetration. The closely related SYP122 is not required for penetration resistance. For the proper study we need the secondary structure of the syntaxin protein which helps us for the better understanding of the given of the plant immune responses. The physical properties of the protein help us to understand the exact structure. On the phylogenetic analysis by using clustal X software we find the observation:

- *Machiloides banksi* and *Pedetontus saltator* are evolved from same ancestor and the showing the root distance 0.006360 . The physiological study shows that *Machiloides banksi* have 78.98 % alpha helix ,extended strand 5.73 % ,  $\beta$ - tern 1.27 % ,Random coil 14.01 % respectively . While *Pedetontus saltator* 80.25 % alpha helix ,extended strand 6.37 % ,  $\beta$ - tern 1.27 % ,Random coil 12.10 % respectively. The physiological properties show that both plant syntaxin have nearly same secondary structure.

- *Scolopendra polymorpha* and *Nicotiana tobacum* are evolved from same ancestor and the shows 0.859760. the physiological study shows that *Scolopendra polymorpha* have 76.22 % alpha helix ,extended strand 4.46 % ,  $\beta$ - tern 1.22% ,Random coil 14.65 % respectively . While *Nicotiana tobacum* 72.00 % alpha helix , extended strand 4.78 % ,  $\beta$ - tern 1.84% ,Random coil 20.96 % respectively. Here the both belongs to same group but the percent of random coil is differs from each other.

- *Neogonodactylus oerstedii* and *Skogsbergia lernerii* are evolved from same ancestor and the shows pair distances of 0.140120. The physiological study shows that *Neogonodactylus oerstedii* have 80.89 % alpha helix ,extended strand 4.46 % ,  $\beta$ - tern 1.27 % ,Random coil 13.38 % respectively . While *Skogsbergia lernerii* 81.53 % alpha helix , extended strand 5.73

% ,  $\beta$ - tern 1.27 % ,Random coil 11.46 % respectively. The physiological properties shows that both plant syntaxin have nearly same secondary structure.

- *Peripatoides novaezealandiae* and *Peripatus* sp. 'Pep' are evolved from same ancestor and the shows pair distances of 0.001910 . The physiological study shows that *Peripatoides novaezealandiae* have 78.34 % alpha helix ,extended strand 5.10 % ,  $\beta$ - tern 1.27 % ,Random coil 15.29 % respectively . While *Peripatus* sp. 'Pep' 79.60 % alpha helix, extended strand 5.10 % ,  $\beta$ - tern 1.27 % , Random coil 14.01 % respectively. The physiological properties show that both plant syntaxin have nearly same secondary structure.

- *Phrynus marginemaculatus* and *Stenochrus portoricensis* are evolved from same ancestor and the shows pair distances of 0.006500 . The physiological study shows that *Phrynus marginemaculatus* have 75.80 % alpha helix ,extended strand 6.37 % ,  $\beta$ - tern 1.27 % ,Random coil 16.56 % respectively . While *Stenochrus portoricensis* 77.92 % alpha helix, extended strand 5.84 % ,  $\beta$ - tern 1.30 % , Random coil 14.94 % respectively. The physiological properties show that both plant syntaxin have nearly same secondary structure.

- *Nicoletia meinerti* and *Periplaneta americana* are evolved from same ancestor and the shows pair distances of 0.038220 . The physiological study shows that *Nicoletia meinerti* have 77.71 % alpha helix ,extended strand 5.10 % ,  $\beta$ - tern 1.27 % ,Random coil 15.92 % respectively . While *Periplaneta americana* , 78.98 % alpha helix, extended strand 5.73 % ,  $\beta$ - tern 1.27 % , Random coil 14.01 % respectively. The physiological properties show that both plant syntaxin have nearly same secondary structure. There are other plant syntaxin are present which shows the different ancestor they physiological properties are as follows. *Tomocerus* sp. 'Tom2 shows the root distances 0.048260, The physiological study shows that *Tomocerus* sp. 'Tom2 have 79.62 % alpha helix ,extended strand 5.73 % ,  $\beta$ - tern 1.27 % ,Random coil 13.38 % respectively, *Scutigereilla* sp. 'Scu3' shows the root distances 0.112690, The physiological study shows that *Scutigereilla* sp. 'Scu3'have 79.62 % alpha helix ,extended strand 4.46 % ,  $\beta$ - tern 1.27 %

,Random coil 14.65 % respectively, *Streptocephalus seali* shows the root distances 0.086410, The physiological study shows that *Streptocephalus seali* 'have 74.52 % alpha helix ,extended strand 5.73 % ,  $\beta$ - tern 1.27 % ,Random coil 18.47 % respectively. *Prokoenenia wheeleri* shows the root distances 0.119220, The physiological study shows that *Prokoenenia wheeleri* 'have 76.58 % alpha helix ,extended strand 5.70 % ,  $\beta$ - tern 1.27 % ,Random coil 16.46 % respectively, *Polyxenus fasciculatus* shows the root distances 0.0218920, The physiological study shows that *Polyxenus fasciculatus* 'have 79.62 % alpha helix ,extended strand 5.10 % ,  $\beta$ - tern 1.27 % ,Random coil 14.01 % respectively.

### Discussion

Vesicular transport is an integral process in eukaryotic cells and the syntaxin, a member of the SNARE protein superfamily, are a critical piece of the vesicular transport machinery. We have obtained syntaxin homologues from determined the paralogue affinity of the homologues by molecular phylogenetics and compared from 17 different plant species which have the different properties . Phylogenetic analysis confirms and extends previous conclusions that the syntaxin families are present in diverse plant species and the syntaxin sub-families diverged early in plant evolution. This result is expanded with the inclusion of new homologues for previously sampled taxa, newly sampled taxa, and newly sampled syntaxin sub-families. Because of their integral role in membrane trafficking, the syntaxin genes represent a valuable potential molecular marker for the experimental study of the plant identification system and plant resistance against the of disease-causing parasite .the protein sequences is highly conserved it means that their very low rate of mutation during evolution so there is less rate of mutation in their gene sequences which helps to make them a good candidates for identification. To different group sequence shows enough variability in their sequences to discriminate between two species though the day belongs.

## References

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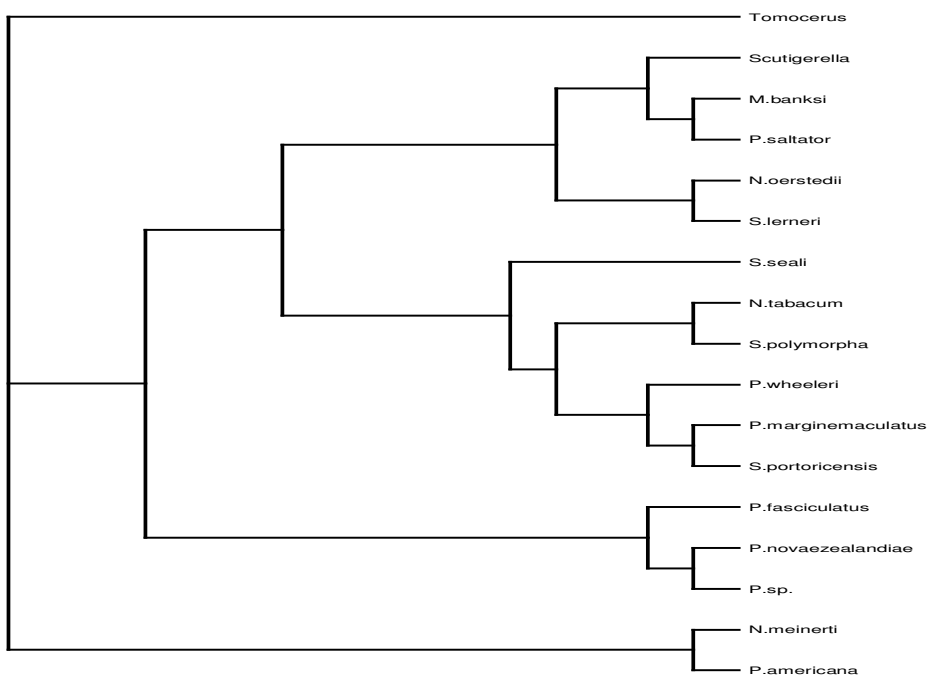
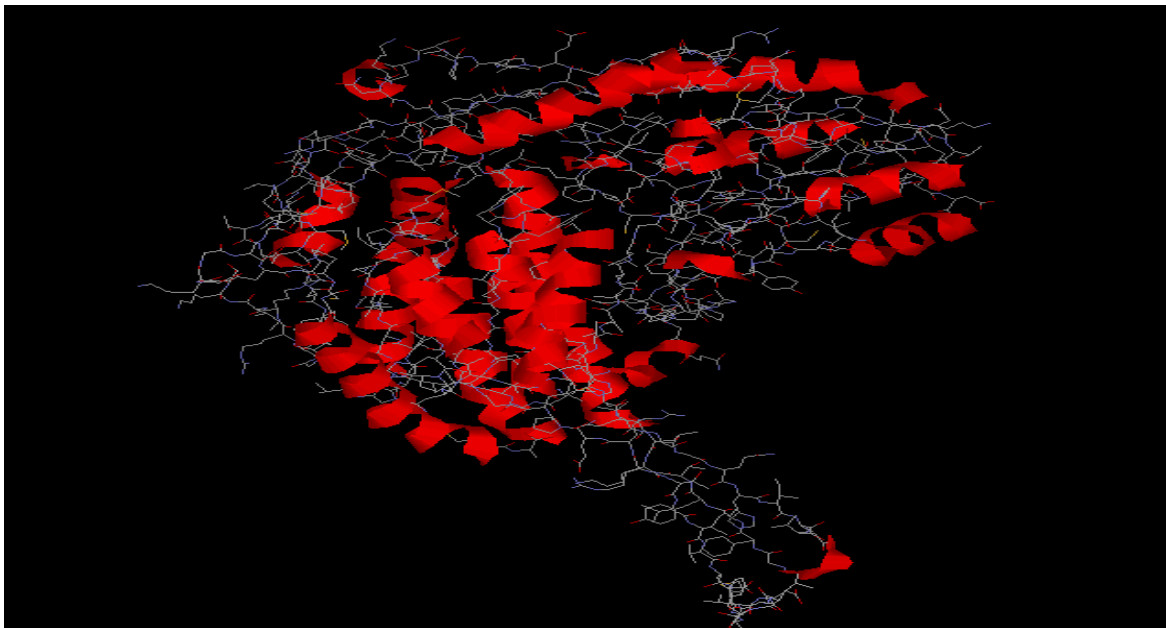


Fig.1- Phylogenetic tree of Syntaxin protein from different plant species showing the species level variation in the protein in different plant species.

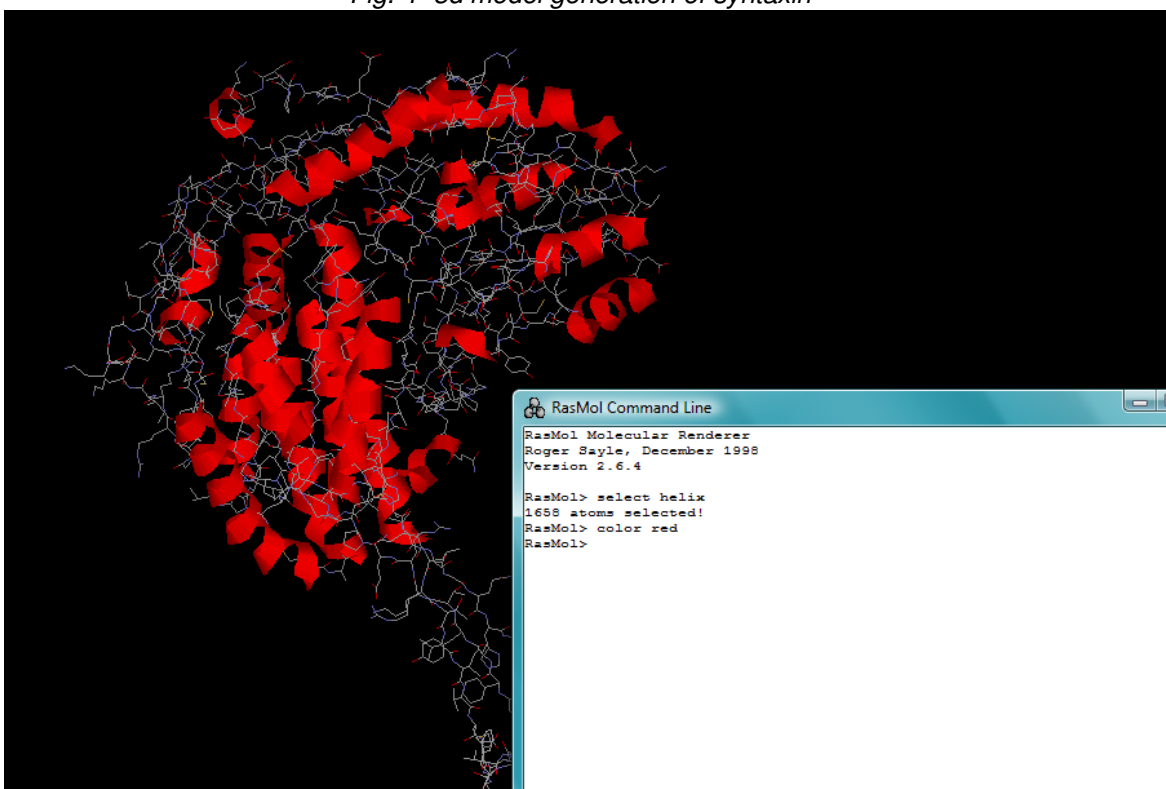
Table 1 -Showing the root distances of all the 17 plant species with respective to syntaxin protein

Plant Name	Root distances
<i>Machiloides banksi</i>	0.08995
<i>Pedetontus saltator</i>	0.08995
<i>Scutigera sp. 'Scu3'</i>	0.11269
<i>Scolopendra polymorpha</i>	0.17792
<i>Nicotiana tabacum</i>	0.76862
<i>Neogonodactylus oerstedii</i>	0.11494
<i>Skogsbergia lernerii</i>	0.08658
<i>Streptocephalus seali</i>	0.08641
<i>Phrynus marginemaculatus</i>	0.09791
<i>Stenochrus portoricensis</i>	0.010183
<i>Prokoenenia wheeleri</i>	0.11922
<i>Peripatoides novaezealandiae</i>	0.06888
<i>Peripatus sp. 'Pep'</i>	0.06251
<i>Polyxenus fasciculatus</i>	0.02189
<i>Nicoletia meinerti</i>	0.02105
<i>Periplaneta americana</i>	0.02257
<i>Tomocerus sp. 'Tom2'</i>	0.04826





*Fig. 4- 3d model generation of syntaxin*



*Fig. 5- 3d Model prediction via structural commands*