

# Development of Molecular Markers for Stress Response in Different Species of Entomopathogenic Nematodes



**Vishal Somvanshi, Hinanit Koltai\* & Itamar Glazer**

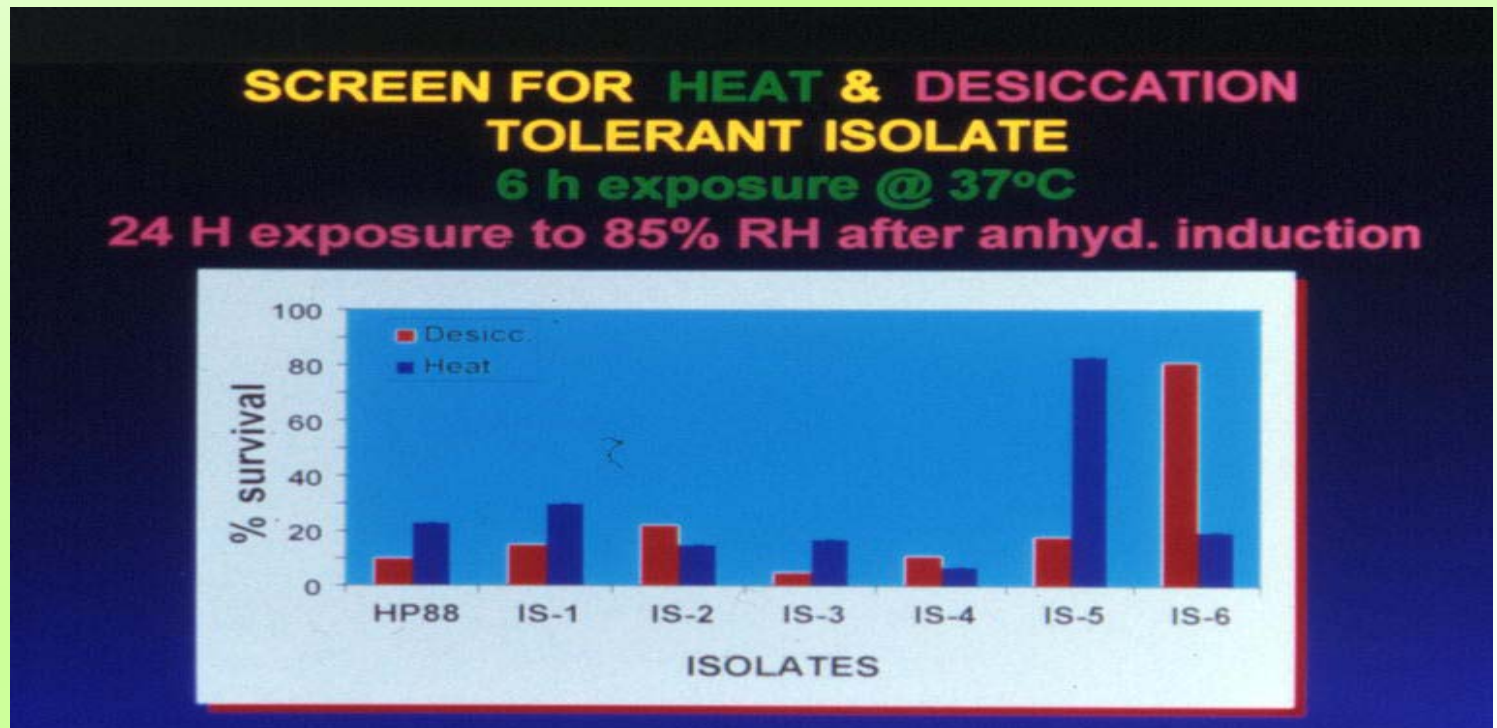
Department of Nematology, \* Ornamental Horticulture

ARO-Volcani Center, Bet Dagan 50250

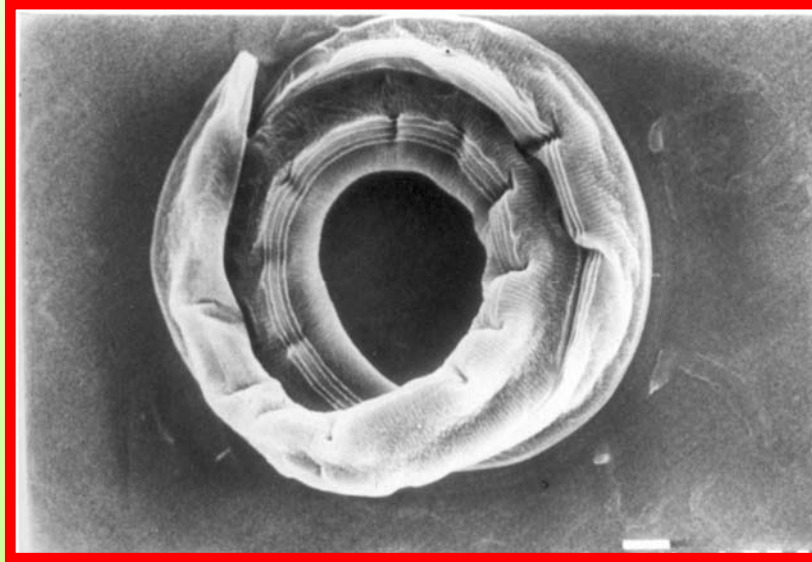
**ISRAEL**

# THE BACKGROUND

- Different entomopathogenic nematode (EPN) species have different stress tolerance capabilities

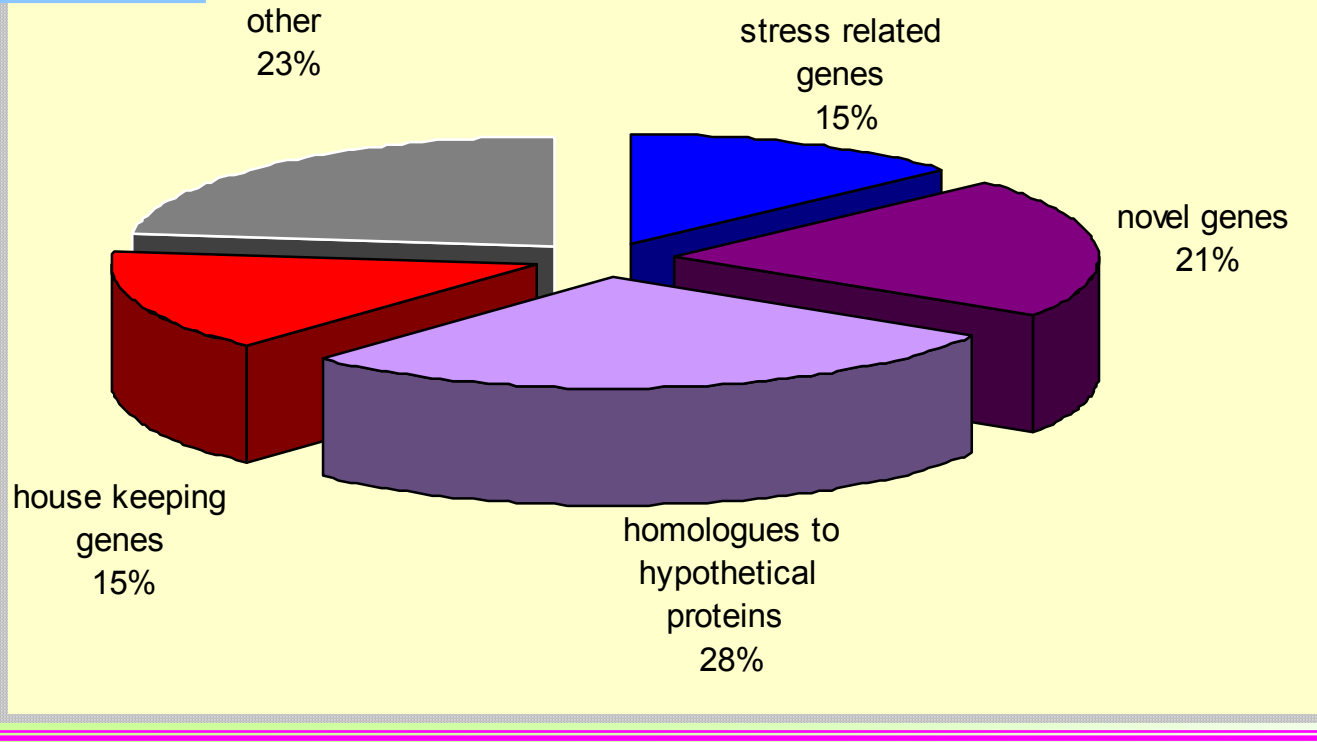
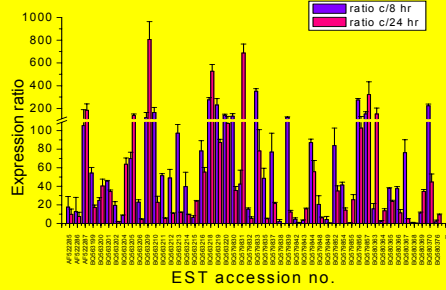


- Molecular studies of desiccation tolerance in EPNs are few



- In one such study (Gal et al., 2003) reported differential up-regulation of a set of genes in *Steinernema feltiae* IS-6 strain

# ESTs in *S. feltiae*



Gal, T. Z., Glazer, I. & Koltai, H. (2003). Differential gene expression during desiccation stress in the insect-killing nematode *Steinernema feltiae* IS-6. *J. Parasitol.* 2003 Aug;89(4):761-6.

# THE QUESTIONS

Same genes expressed alike across different EPN species?

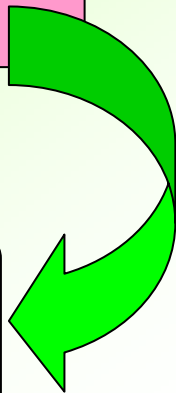
YES

Markers for desiccation !

NO

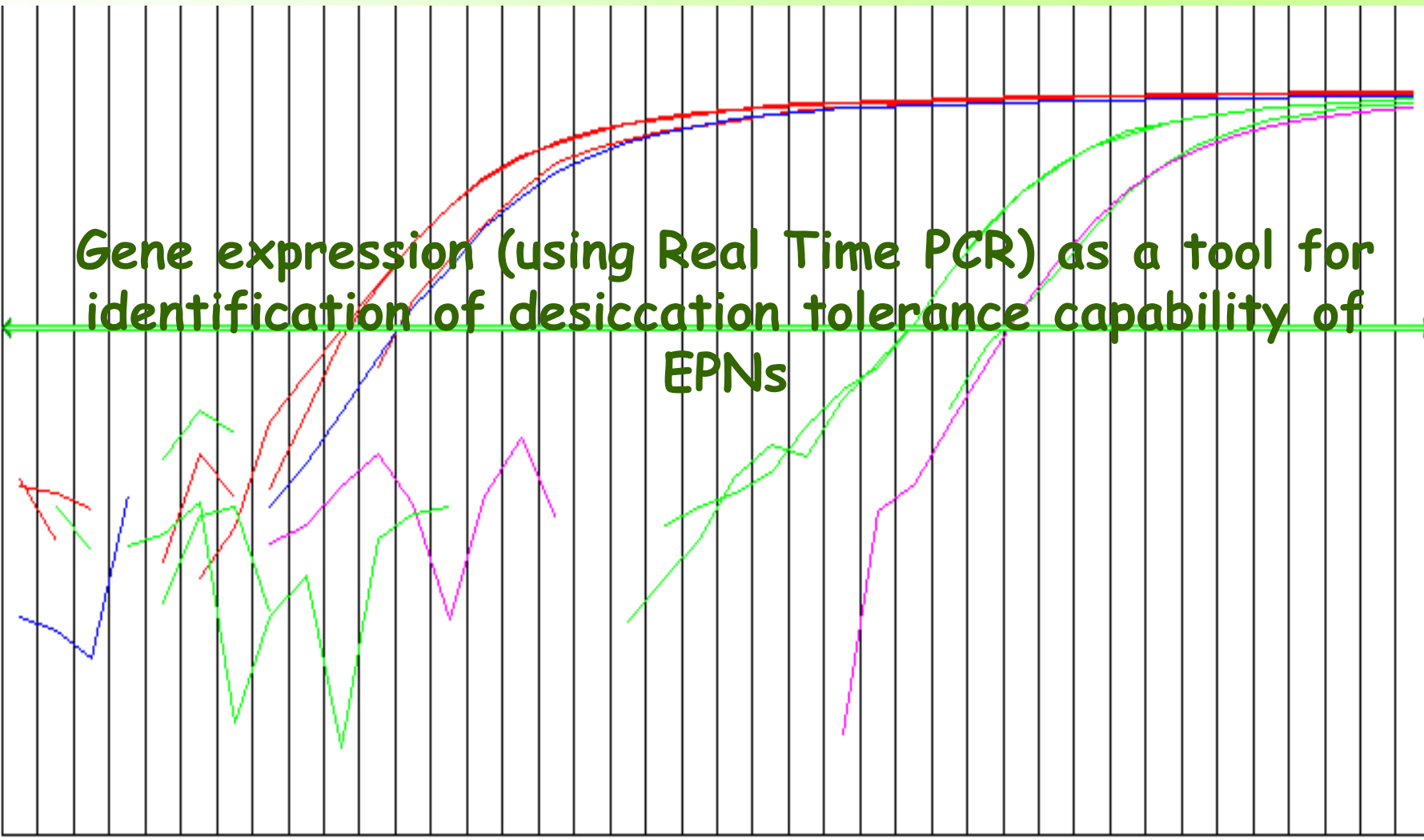
A fingerprint for desiccation tolerance capability of EPNs?

Use as marker for characterization of hardy EPNs & in EPN genetic improvement?



# THE IDEA

Gene expression (using Real Time PCR) as a tool for identification of desiccation tolerance capability of EPNs



# EXPERIMENTAL MATERIALS-

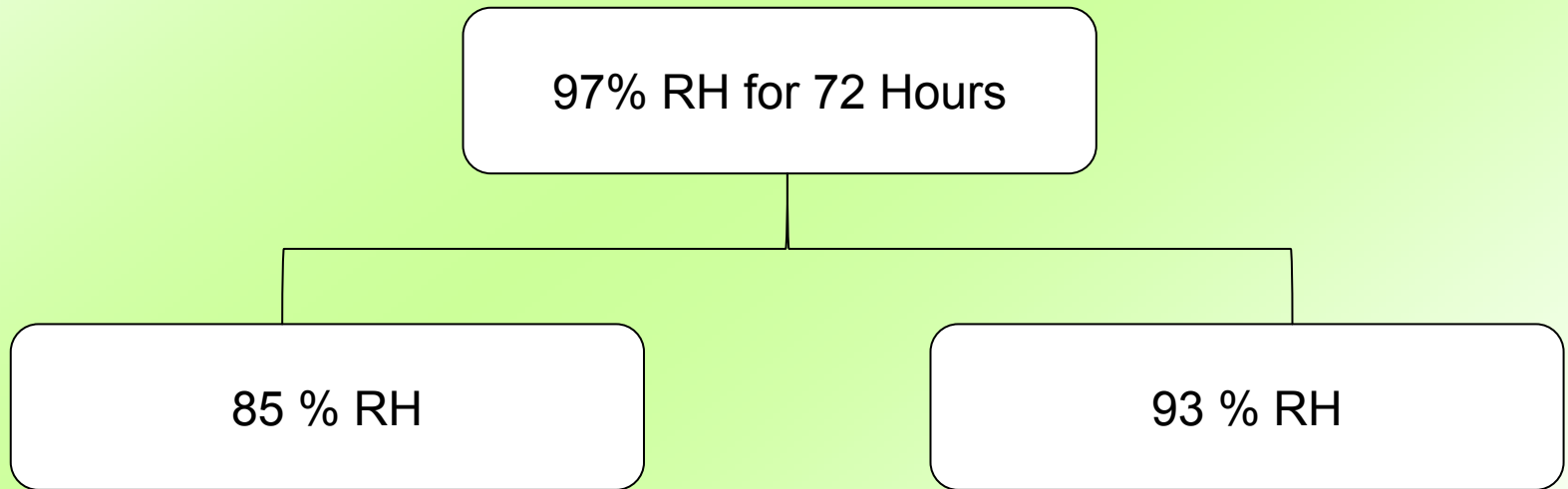
## the nematodes

1. *Steinernema feltiae* IS-6 (SFG)
2. *S. feltiae* Carmiel strain (SFC)
3. *S. carpocapsae* Mexican strain (SCM) (Clade II)
4. *S. riobrave* (SR) (Clade IV)
5. *Heterorhabditis bacteriophora* (Strain TTO1)

# METHODOLOGY

Two parallel experiments- Biological desiccation assays and Molecular investigations

## 1. Bio-Assays



Nematode survival & mortality counted at 24, 48, 72, 96, 120 and 144 h

# METHODOLOGY- 2.Molecular analysis

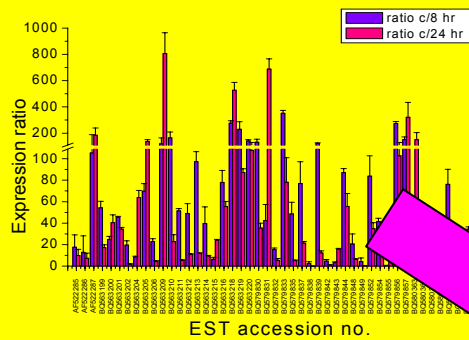
Nematode desiccation for 8,16,24 & 32h  
(Solomon et al.,1999)

RNA isolation

Reverse Transcription  
& Real time qPCR

Results and Analysis

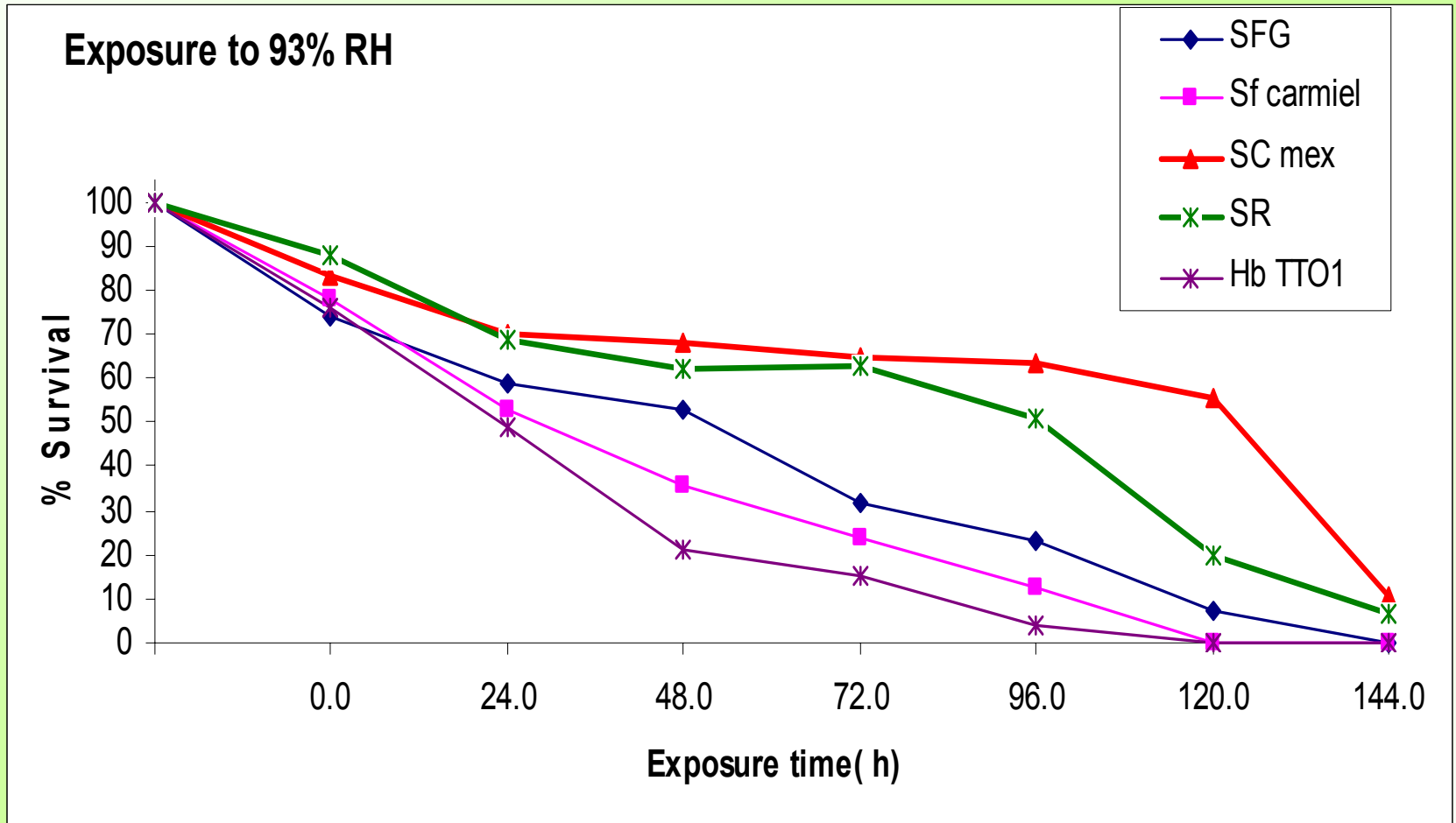
# Genes studied



1. Aldehyde dehydrogenase (*Sf-Aldh*)
2. Heat Shock Protein 40 (*Hsp40*)
3. Glutathion Peroxidase (*Glp*)
4. Nucleosome Binding Protein (*Nap1*)
5. Heat Shock Protein 60 (*Hsp60*)

18s rRNA Gene as internal reference

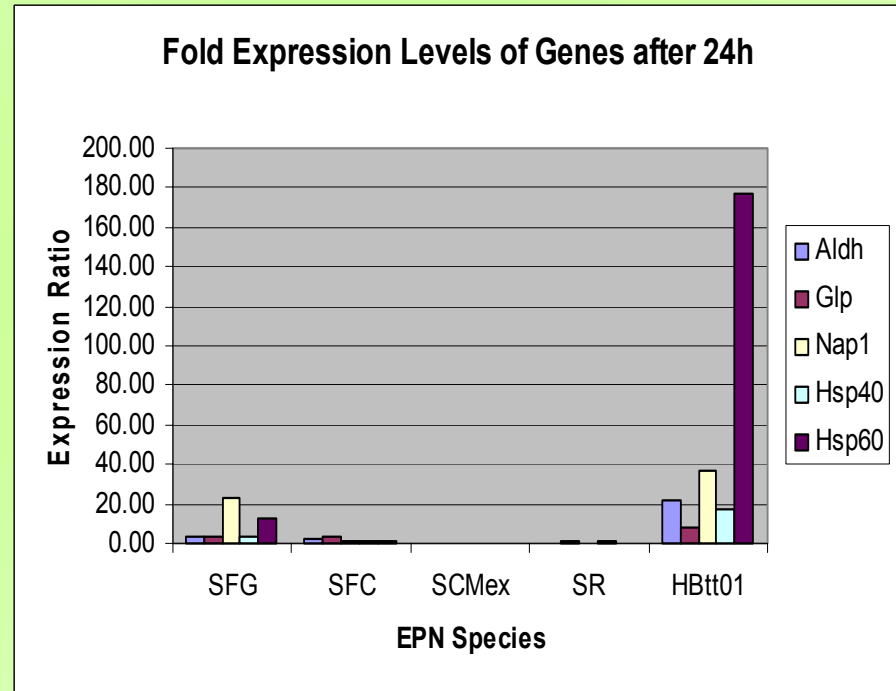
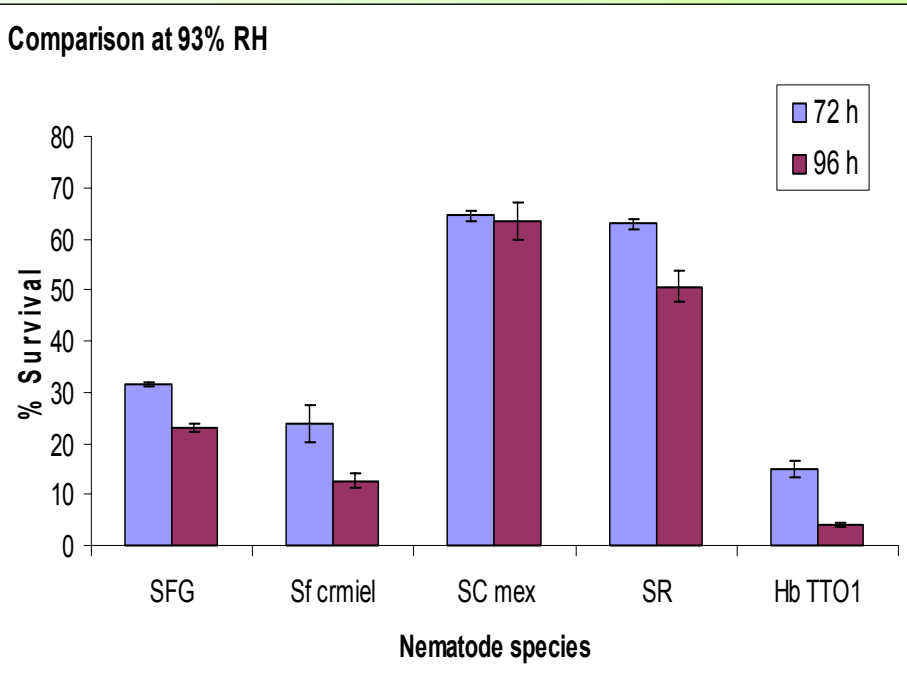
# EXPERIMENTAL RESULTS OF BIOASSAYS



# EXPERIMENTAL RESULTS OF QPCR

Nematodes Genes	<i>S. feltiae</i> (IS-6)	<i>S. feltiae</i> (Carmel)	<i>S.</i> <i>Carpocapsae</i> ( Mexican)	<i>S.</i> <i>riobrave</i>	<i>H.</i> <i>bacteriophoro</i>
<i>Aldh</i>	3.48 ↑	1.76 ↑	0.31 ↓	0.45 ↓	22.19 ↑
<i>Glp</i>	2.94 ↑	3.40 ↑	0.53 ↓	0.76 ↓	7.71 ↑
<i>Nap1</i>	22.88 ↑	0.93 ↓	0.36 ↓	0.33 ↓	37.36 ↑
<i>Hsp40</i>	3.45 ↑	1.08 ↑	0.24 ↓	0.71 ↓	17.11 ↑
<i>Hsp60</i>	12.30 ↑	0.8 ↓	0.15 ↓	0.11 ↓	176.6 ↑

# RESULTS- THE CORRELATION



## Results contd..

- *Desiccation survival rankings for the nematodes were SR>SCM>SFC>SFG>Hb*
- *H. bacteriophora tt01* showed highest expression ratios for all genes, followed by *S. feltiae IS-6(SFG)* and *S. feltiae Carmiel* strain, respectively.
- *S. riobrave* and *S. carpocapsae Mexican Strain* showed least expression ratios for all genes.

# CONCLUSIONS

- Every EPN species had unique expression pattern for different genes
- Gene expression using Real time PCR could be effectively used to develop molecular markers for desiccation tolerance capability in EPNs

- **Inverse correlation between gene expression and desiccation tolerance capability of EPNs.**
- **Shutdown of genes (or metabolism?)**

**the quicker the EPN shuts down its genes- the better desiccation survivor it is !**

# FUTURE DIRECTIONS

- Using these genes in Marker Assisted Selection (MAS) for desiccation tolerance in EPN genetic improvement
- Profiling desiccation tolerance capability of wild populations of EPN.
- Global analysis of gene expression using *C. elegans* chips
- A key to other similar studies where markers would have more crucial role

# ACKNOWLEDGEMENTS

- Ministry of Foreign Affairs, Government of Israel for Post Doctoral Fellowship (2005-06)
- Liora

## Personally..

- Dr. Itamar Glazer
- Dr. Hinanit Koltai
- Dr. Ralf Ehlers
- COST 850

THANKS TO YOU ALL