|  |  |
| --- | --- |
| **Primer** | **Sequence** |
| g1 sense -F | TCTGCAGGATCCGATGGTGGCGAATGTTCAAC |
| g1 sense -R | TCTGCACTCGAGCCTTCCTCATCGACAGAAAGTG |
| g1 antisense -F | TCTGCAGAGCTCGATGGTGGCGAATGTTCAAC |
| g1 antisense - R | TCTGCAGGTACCCCTTCCTCATCGACAGAAAGTG |
| Mi 18S QRT F 1 | GGCTCATGGTGGAAAGTATG |
| Mi 18S QRT R 1 | CCCCAGTGTAATGTCCTTTG |
| Mi-actin\_F | GCTTTGCTATGTTGCTTTGG |
| Mi-actin\_R | TGTAAGAAGTCTCGTGAATACC |
| qGlp2\_F | CGTTGTCTCTTTGATGGTTTCG |
| qGlp2\_R | CTGATCGCATTGCCCATTTG |
| G78/260rB | CGGGAAAAGTTCTTCTGGTTGT |
| G78/260rB | AAATGCCCTCGACTAGCTGA |

**Table S1.** List of primers used in the study.

**Table S2.** Comparative gene sizes of *glp-1* in different nematode species.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Organism** | **Accession** | **Gene** | **Transcript** | **Protein size** |
|  | **number** | **size (kb)** | **size (kb)** | **(amino acids)** |
| *Caenorhabditis elegans* | CAA79620.1 | 7.45 | 4.32 | 1295 |
| Caenorhabditis briggsae | CBG06809 | 6.61 | 4.43 | 1326 |
| Caenorhabditis japonica | CJA09628 | 11.48 | 3.94 | 1294 |
| Caenorhabditis remanei | CRE29209 | 6.12 | 4.32 | 1308 |
| *Meloidoyne incognita* | MiV1ctg1087 | 7.44 | 3.25 | 1083 |
| *Meloidogyne hapla* | Mh10g200708\_Contig1018 | 5.8 | 2.27 | 757 |
| *Globodera pallida* | GPLIN\_000999900.1 | N.A. | 3.75 | 1,250 |
| *Meloidogyne floridensis* | contig nMf\_1\_1\_scaf00321 | N.A. | 2.69 | 898 |

**Table S3.** Size evaluation of females obtained from wild-type plants and transgenic RNAi lines.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Wild type** | | **RNAi line** | |
| **Female** | **Length** | **Breadth** | **Length** | **Breadth** |
|  | **(µm)** | **(µm)** | **(µm)** | **(µm)** |
| 1 | 343 | 206 | 249 | 180 |
| 2 | 442 | 299 | 181 | 130 |
| 3 | 243 | 295 | 263 | 164 |
| 4 | 530 | 368 | 234 | 142 |
| 5 | 462 | 350 | 253 | 135 |
| 6 | 515 | 308 | 213 | 111 |
| 7 | 498 | 342 | 183 | 114 |
| 8 | 443 | 338 | 287 | 136 |
| 9 | 488 | 233 | 242 | 124 |
| 10 | 355 | 196 | 267 | 187 |
| 11 | 374 | 390 | 179 | 121 |
| Average | 426.64 | 302.273 | 231.9 | 140.36 |

**Table S4A.** Numbers of eggs counted and calculated MF value for *M. incognita* feeding on wild-type plants and RNAi plants.

|  |  |  |
| --- | --- | --- |
| **number of egg masses** | | |
|  | **wild** | **Transgenic** |
|  | **type** | **line 1** |
| 1 | 87 | 45 |
| 2 | 125 | 39 |
| 3 | 102 | 50 |
| 4 | 117 | 54 |
| 5 | 92 | 57 |
| 6 | 96 | 37 |
| 7 | 114 | 80 |
| 8 | 100 | 53 |
| 9 | 89 | 82 |
| 10 | 109 | 29 |
| Average | 103.1 | 52.6 |

|  |  |  |
| --- | --- | --- |
| **Number of eggs per egg mass** | | |
|  | **wild type** | **Transgenic 1** |
| 1 | 283 | 188 |
| 2 | 307 | 213 |
| 3 | 337 | 250 |
| 4 | 326 | 168 |
| 5 | 335 | 256 |
| 6 | 318 | 245 |
| 7 | 239 | 148 |
| 8 | 314 | 217 |
| 9 | 264 | 278 |
| 10 | 277 | 237 |
| Avg | 300 | 220 |

|  |  |
| --- | --- |
| **Estimated multiplication factor (MF)** | |
| No. of egg masses\* No. of eggs per egg mass/initial nematode inoculum | |
| **Wild type** | 103\*300/1000 |
|  | 30.93 |
|  |  |
| Transgenic | 52.6\*220/1000 |
|  | 11.572 |

|  |  |
| --- | --- |
| **Null Hypothesis:** | **H0: μ1 = μ2 = μ3 ... = μk** |
| **Hypothesis:** | **H1: Means are not all equal.** |

**Table S4A. cont….**

**Anova: Two-Factor Without Replication**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |
| Row 1 | 2 | 471 | 235.5 | 4512.5 |
| Row 2 | 2 | 520 | 260 | 4418 |
| Row 3 | 2 | 587 | 293.5 | 3784.5 |
| Row 4 | 2 | 494 | 247 | 12482 |
| Row 5 | 2 | 591 | 295.5 | 3120.5 |
| Row 6 | 2 | 563 | 281.5 | 2664.5 |
| Row 7 | 2 | 387 | 193.5 | 4140.5 |
| Row 8 | 2 | 531 | 265.5 | 4704.5 |
| Row 9 | 2 | 542 | 271 | 98 |
| Row 10 | 2 | 514 | 257 | 800 |
|  |  |  |  |  |
| Column 1 | 10 | 3000 | 300 | 1074.9 |
| Column 2 | 10 | 2200 | 220 | 1716 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Rows | 16393 | 9 | 1821.4 | 1.8789 | 0.180650858 | 3.178893105 |
| Columns | 32000 | 1 | 32000 | 33.009 | 0.000277934 | 5.117355008 |
| Error | 8725 | 9 | 969.44 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 57118 | 19 |  |  |  |  |

F>Fcr; Hence, null hypothesis is rejected. Therefore diffrenece between means of samples.

**t-Test: Two-Sample Assuming Equal Variances**

|  |  |
| --- | --- |
| **Null Hypothesis:** | **H0: No significance in the difference of means** |
| **Hypothesis:** | **H1: There is a significant difference in means** |

**Table S4A. cont….**

|  |  |  |
| --- | --- | --- |
|  | *Variable 1* | *Variable 2* |
| Mean | 300 | 220 |
| Variance | 1074.8889 | 1716 |
| Observations | 10 | 10 |
| Pooled Variance | 1395.4444 |  |
| Hypothesized Mean Difference | 0 |  |
| Df | 18 |  |
| t Stat | 4.7887119 |  |
| P(T<=t) one-tail | 7.349E-05 |  |
| t Critical one-tail | 1.7340636 |  |
| P(T<=t) two-tail | 0.000147 |  |
| t Critical two-tail | 2.100922 |  |

 Two-tailed test: lf t Stat < -t Critical two-tail or t Stat > t Critical two-tail, we reject the null hypothesis.

Therefore, there is a significant difference in the means of the two samples

**Table S4B.** Statistical analyses of phenotypes of J2s obtained from *M. incognita* females isolated from infected RNAi and wild-type plants.

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Stylet length** | **Length from Mouth** |
|  |  |  | **to metacorpus** |
| **wild type J2s** | 1 | 10 | 45 |
|  | 2 | 14 | 47 |
|  | 3 | 11 | 46 |
|  | 4 | 11 | 44 |
|  | 5 | 16 | 54 |
|  | 6 | 11 | 58 |
|  | 7 | 11 | 47 |
|  | 8 | 16 | 65 |
|  | 9 | 14 | 63 |
|  | 10 | 16 | 68 |
| **RNAi J2s** | 1 | 10 | 41 |
|  | 2 | 8 | 35 |
|  | 3 | 9 | 39 |
|  | 4 | 9 | 39 |
|  | 5 | 11 | 42 |
|  | 6 | 10 | 45 |
|  | 7 | 10 | 42 |
|  | 8 | 14 | 45 |
|  | 9 | 15 | 56 |
|  | 10 | 13 | 46 |

|  |  |
| --- | --- |
| **Null Hypothesis:** | **H0: μ1 = μ2 = μ3 ... = μk** |
| **Hypothesis:** | **H1: Means are not all equal.** |

**Table S4B. cont….**

**Anova: Two-Factor With Replication**

|  |  |  |  |
| --- | --- | --- | --- |
| SUMMARY | Stylet | Length from | Total |
|  | length | Mouth to metacorpus |  |
| *wild type J2s* |  |  |  |
| Count | 10 | 10 | 20 |
| Sum | 130 | 537 | 667 |
| Average | 13 | 53.7 | 33.35 |
| Variance | 6 | 84.011 | 478.56 |
|  |  |  |  |
| *RNAi J2s* |  |  |  |
| Count | 10 | 10 | 20 |
| Sum | 109 | 430 | 539 |
| Average | 10.9 | 43 | 26.95 |
| Variance | 5.4333 | 32 | 288.89 |
|  |  |  |  |
| *Total* |  |  |  |
| Count | 20 | 20 |  |
| Sum | 239 | 967 |  |
| Average | 11.95 | 48.35 |  |
| Variance | 6.5763 | 85.082 |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **ANOVA** |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Sample | 409.6 | 1 | 409.6 | 12.856 | 0.001 | 4.1132 |
| Columns | 13250 | 1 | 13250 | 415.85 | 2E-21 | 4.1132 |
| Interaction | 184.9 | 1 | 184.9 | 5.8033 | 0.0212 | 4.1132 |
| Within | 1147 | 36 | 31.861 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 14991 | 39 |  |  |  |  |

F>Fcr; Hence, null hypothesis is rejected. Therefore diffrenece between means of samples.

**Table S4B. cont….**

**Student's T test**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Wild J2s** | **RNAi J2s** |  | **Wild J2s** | **RNAi J2s** |
| **Stylet** | 10 | 10 | **Length from Mouth** | 45 | 41 |
| **length** | 14 | 8 | **to metacorpus** | 47 | 35 |
|  | 11 | 9 |  | 46 | 39 |
|  | 11 | 9 |  | 44 | 39 |
|  | 16 | 11 |  | 54 | 42 |
|  | 11 | 10 |  | 58 | 45 |
|  | 11 | 10 |  | 47 | 42 |
|  | 16 | 14 |  | 65 | 45 |
|  | 14 | 15 |  | 63 | 56 |
|  | 16 | 13 |  | 68 | 46 |

|  |  |
| --- | --- |
| **Null Hypothesis:** | **H0: No significance in the difference of means** |
| **Hypothesis:** | **H1: There is a significant difference in means** |

**t-Test: Two-Sample Assuming Equal Variances**

**Stylet length**

|  |  |  |
| --- | --- | --- |
|  | *Variable 1* | *Variable 2* |
| Mean | 13 | 10.9 |
| Variance | 6 | 5.4333 |
| Observations | 10 | 10 |
| Pooled Variance | 5.716666667 |  |
| Hypothesized Mean Difference | 0 |  |
| Df | 18 |  |
| t Stat | 1.963961012 |  |
| P(T<=t) one-tail | 0.032584743 |  |
| t Critical one-tail | 1.734063592 |  |
| P(T<=t) two-tail | 0.065169487 |  |
| t Critical two-tail | 2.100922037 |  |

**Table S4B. cont….**

**Length from Mouth to metacarpus**

**t-Test: Two-Sample Assuming Equal Variances**

|  |  |  |
| --- | --- | --- |
|  | *Variable 1* | *Variable 2* |
| Mean | 53.7 | 43 |
| Variance | 84.011 | 32 |
| Observations | 10 | 10 |
| Pooled Variance | 58.006 |  |
| Hypothesized Mean Difference | 0 |  |
| Df | 18 |  |
| t Stat | 3.1415 |  |
| P(T<=t) one-tail | 0.0028 |  |
| t Critical one-tail | 1.7341 |  |
| P(T<=t) two-tail | 0.0056 |  |
| t Critical two-tail | 2.1009 |  |

 Two-tailed test; lf t Stat < -t Critical two-tail or t Stat > t Critical two-tail, we reject the null hypothesis.