**Control of western corn rootworm via RNAi traits in maize: lethal and sublethal effects of *Sec23* dsRNA**

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**SUPPLEMENTARY TABLES**

**Supplementary Table 1.** Primer sequences used to quantify transcript knockdown in WCR adults.

|  |  |  |
| --- | --- | --- |
| **Gene name** | **Primer sequences for RT-qPCR** | **Product Length (bp)** |
| *Sec23* | Forward: AGCTCCATTCAACCTGACAGA  Reverse: TGTGCATCATCTACTGGAGCC | 161 |
| *Sec23* (plant experiments) | Forward: TGCAAACTAGGTTCCCAATG  Reverse: TATGCTGTGGACGAAACTGC | 211 |
| *β*-actin | Forward: TCCAGGCTGTACTCTCCTTG  Reverse: CAAGTCCAAACGAAGGATTG | 134 |

**Supplementary Table 2.** Primer sequences used to quantify transcript knockdown in WCR larvae.

|  |  |  |
| --- | --- | --- |
| **Gene name** | **Primer sequences for RT-qPCR** | **Product Length (bp)** |
| *β-tubulin* | Forward: TTGAGTTGCCGATGAAAGTG  Reverse: GATCCCAGACACGGAAGGTA | 205 |
| *β-actin* | Forward: TCCAGGCTGTACTCTCCTTG  Reverse: CAAGTCCAAACGAAGGATTG | 134 |
| *Sec23* | Forward: CTGTTGTTGCACCAGGAAGC  Reverse: CATAACTCGGGCGCCAGTAT | 200 |

**Supplementary Table 3**. Mortality and growth inhibition of WCR larvae after nine days of feeding with *Sec23* dsRNA. GC = Growth Inhibition; SEM = standard error of the mean; Replicates = 8 wells per replicate, 2-3 insects per well. Means were separated using the Tukey-Kramer test in JMP Pro. Letters in parentheses designate statistical levels: levels with different letters are significantly different (*p* < 0.05).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment/**  **dsRNA** | **Concentration (ng/cm2)** | **Replicates** | **% mortality**  **± SEM** | **GC ± SEM** |
| *Sec23* | 500 | 6 | 53.0 ± 12.8 **(A)** | 0.56 ± 0.27 **(A)** |
| *Sec23* v1 | 500 | 6 | 66.1 ± 4.8 **(A)** | 0.85 ± 0.03 **(A)** |
| *Sec23* v2 | 500 | 6 | 65.7 ± 10.0 **(A)** | 0.84 ± 0.04 **(A)** |
| 0.1X TE | 0 | 12 | 10.4 ± 3.3 **(B)** | -0.04 ± 0.05 **(B)** |
| water | 0 | 12 | 8.4 ± 2.5 **(B)** | 0.08 ± 0.03 **(B)** |
| *YFP* dsRNA | 500 | 12 | 9.6 ± 2.5 **(B)** | 0.10 ± 0.05 **(B)** |

**SUPPLEMENTARY FIGURE**

**![A close up of a map

Description automatically generated]()**

**Supplementary Figure 1. Effect of a single *Sec23* dsRNA exposure in WCR adults. A.** Adult mortality after a single *Sec23* dsRNA (383 bp, Supplementary Sequence 3) feeding for 48 h at 50 and 100 ng/diet plug; artificial diet and 100 ng of *GFP* dsRNA were used as controls. **B.** Adult mortality after single *Sec23* dsRNA (383 bp, Supplementary Sequence 3) feeding for 3 h and 6 h at 50 ng/diet plug; artificial diet and 100 ng of *GFP* dsRNA were used as controls. Error bars indicate standard errors of the mean from three biological replicates (*i.e.*, three generations) of ten mixed sex WCR adults (~48 h old) per treatment for a total of 30 insects per treatment.

**SUPPLEMENTARY SEQUENCES**

**Sequence 1**

> *D. v. virgifera* Sec23 protein

MSTYEEYIQQNEDRDGIRFTWNVWPSSRIEATRLVVPLACLYQPIKERLDLPPIQYDPVLCTRNTCRAILNPLCQVDYRAKLWVCNFCFQRNPFPPQYAAISEQHQPAELMPMFSTIEYTITRAQCLPPIFLYVVDTCMDDEELGSLKDSLQMSLSLLPPNALIGLITFGKMVQVHELGTEGCSKSYVFRGTKDLSAKQVQEMLGIGKVALGQQAPQQPGQPLRPGQMQPTVVAPGSRFLQPVSKCDMNLTDLIGEQQKDPWPVHQGKRYLRSTGVALSIAIGLLECTYSNTGARVMLFVGGPCSQGPGQVVNDDLKQPIRSHHDIQKDNAKYMKKGIKHYDALAMRAATNGHSVDIYSCALDQTGLMEMKQCCNSTGGHMVMGDSFNSSLFKQTFQRVFTRDQKSDLKMAFNGTLEVKCSRELKVQGGIGSCVSLNVKSPLVSDTEIGMGNTVQWKMCTLTPSTTMSLFFEVVNQHSAPIPQGGRGCIQFITQYQHSSGQRKIRVTTVARNWADATANIHHISAGFDQEAAAVIMARMAVYRAESDDSPDVLRWVDRMLIRLCQKFGEYNKDDPNSFRLGQNFSLYPQFMYHLRRSQFLQVFNNSPDETSFYRHMLMREDLTQSLIMIQPILYSYSFNGPPEPVLLDTSSIQPDRILLMDTFFQILIFHGETIAQWRSLKYQDMPEYENFRQLLQAPVDDAQEILQTRFPMPRYIDTEQGGSQARFLLSKVNPSQTHNNMYSYGGDSGAPVLTDDVSLQVFMDHLKKLAVSSTA

**Sequence 2**

> *D. v. virgifera Sec23* open reading frame

ATGAGCACATATGAAGAGTATATACAACAAAATGAAGATCGAGATGGGATTAGATTTACCTGGAATGTATGGCCTTCAAGCAGAATTGAAGCTACCCGTCTCGTAGTACCCTTAGCTTGTCTGTACCAGCCTATAAAGGAACGTCTGGATCTTCCACCAATACAATATGACCCTGTTTTATGTACTAGAAATACTTGTAGAGCAATATTAAACCCACTGTGTCAGGTAGATTATCGAGCAAAACTCTGGGTATGCAACTTTTGTTTCCAGAGAAATCCATTTCCACCTCAATATGCTGCTATTTCAGAACAACATCAACCAGCGGAATTGATGCCTATGTTTTCCACCATTGAATACACAATAACTAGAGCTCAATGTTTACCACCAATATTTTTGTATGTTGTTGACACCTGCATGGATGATGAAGAACTGGGTTCCCTGAAAGACTCATTGCAAATGTCCCTTAGTTTGTTGCCACCTAATGCGTTAATAGGACTAATAACATTTGGGAAAATGGTTCAAGTTCATGAACTTGGCACTGAAGGTTGTAGTAAGTCATATGTGTTCAGAGGTACAAAAGATCTTAGTGCTAAACAGGTTCAAGAAATGCTGGGAATAGGCAAAGTGGCTTTAGGTCAGCAAGCCCCTCAACAGCCAGGGCAGCCTCTAAGACCTGGGCAAATGCAACCTACTGTTGTTGCACCAGGAAGCAGGTTTCTACAACCTGTATCCAAATGCGATATGAATCTAACAGACCTAATAGGAGAACAACAGAAAGATCCTTGGCCTGTTCATCAGGGTAAAAGGTATTTAAGATCTACAGGTGTAGCTTTATCGATTGCCATTGGTTTGTTAGAATGTACATATTCCAATACTGGCGCCCGAGTTATGCTATTTGTTGGAGGACCTTGCTCACAAGGACCTGGTCAGGTAGTTAATGATGATTTAAAACAGCCTATTAGATCACATCATGATATTCAGAAAGATAATGCAAAATATATGAAGAAAGGTATTAAACATTATGATGCGTTAGCAATGAGAGCCGCAACTAATGGTCACTCTGTTGATATTTATTCTTGTGCTTTGGATCAGACAGGTCTGATGGAAATGAAGCAATGCTGTAATTCTACTGGGGGACACATGGTAATGGGGGATTCATTTAATTCTTCCTTGTTTAAGCAAACTTTCCAACGTGTGTTTACCAGAGATCAAAAAAGTGATCTGAAAATGGCATTTAACGGTACTTTGGAAGTGAAGTGTTCCCGAGAATTAAAAGTTCAAGGAGGTATCGGTTCGTGTGTATCACTTAACGTGAAGAGCCCCTTGGTTTCCGACACAGAAATAGGAATGGGTAATACTGTGCAATGGAAAATGTGTACTTTAACGCCAAGTACTACCATGTCTTTATTCTTTGAGGTCGTAAATCAACATTCTGCTCCCATACCTCAAGGTGGTAGAGGTTGTATACAATTTATTACGCAGTACCAGCATTCAAGTGGTCAAAGAAAAATCAGAGTAACAACAGTGGCTCGAAATTGGGCTGACGCAACTGCTAATATACACCATATCAGTGCCGGATTCGATCAAGAAGCTGCTGCTGTAATAATGGCTAGGATGGCCGTTTATAGGGCAGAATCTGATGATAGTCCAGATGTTCTTAGATGGGTTGACAGAATGCTGATTAGATTGTGTCAAAAATTCGGAGAATACAATAAGGACGACCCCAATTCATTCAGACTTGGTCAAAACTTCAGTCTTTACCCACAGTTCATGTATCACTTAAGAAGATCTCAATTTCTTCAAGTATTCAATAATTCTCCGGACGAGACTTCATTCTACAGACACATGTTGATGAGGGAAGATCTTACTCAATCTTTGATAATGATTCAACCTATTTTGTATAGTTATAGTTTCAATGGTCCACCAGAGCCTGTATTACTAGATACTAGCTCCATTCAACCTGACAGAATATTACTTATGGATACTTTCTTCCAAATATTAATTTTCCATGGAGAGACTATCGCCCAATGGCGTAGTTTAAAATATCAAGACATGCCAGAATATGAAAACTTTAGACAGCTACTACAGGCTCCAGTAGATGATGCACAAGAAATTTTGCAAACTAGGTTCCCAATGCCGAGATATATTGATACCGAACAAGGCGGATCCCAAGCCAGATTTTTGTTGTCGAAAGTAAATCCAAGTCAAACTCATAACAACATGTATTCCTACGGAGGTGATTCTGGAGCTCCAGTTTTGACAGATGATGTATCCCTTCAAGTATTCATGGACCATCTAAAGAAATTGGCAGTTTCGTCCACAGCATAA

**Sequence 3**

> *D. v. virgifera Sec23* dsRNA

AGGACGACCCCAATTCATTCAGACTTGGTCAAAACTTCAGTCTTTACCCACAGTTCATGTATCACTTAAGAAGATCTCAATTTCTTCAAGTATTCAATAATTCTCCGGACGAGACTTCATTCTACAGACACATGTTGATGAGGGAAGATCTTACTCAATCTTTGATAATGATTCAACCTATTTTGTATAGTTATAGTTTCAATGGTCCACCAGAGCCTGTATTACTAGATACTAGCTCCATTCAACCTGACAGAATATTACTTATGGATACTTTCTTCCAAATATTAATTTTCCATGGAGAGACTATCGCCCAATGGCGTAGTTTAAAATATCAAGACATGCCAGAATATGAAAACTTTAGACAGCTACTACAGGCTCCAGTA

**Sequence 4**

> *D. v. virgifera Sec23 v1* dsRNA

AGGTTCCCAATGCCGAGATATATTGATACCGAACAAGGCGGATCCCAAGCCAGATTTTTGTTGTCGAAAGTAAATCCAAGTCAAACTCATAACAACATGTATTCCTACGGAGGTGATTCTGGAGCTCCAGTTTTGACAGATGATGTATCCCTTCAAGTATTCATGGACCATCTAAAGAAATTGGCAGTTTCGTCCACAGCATAA

**Sequence 5**

> *D. v. virgifera Sec23 v2* dsRNA

ATTCCTACGGAGGTGATTCTGGAGCTCCAGTTTTGACAGATGATGTATCCCTTCAAGTATTCATGGACCATCTAAAGAAATTGGCAGTTTCGTCCACAGCATAA

**Sequence 6**

>*GFP* dsRNA

GGGAGTGATGCTACATACGGAAAGCTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCTCTTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCATATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGAACTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAGTACAACTATAACTCACACAATGTATACATCACGGCAGACAAACAACCCA

**Sequence 7**

>*YFP* dsRNA

caccatgggctccagcggcgccctgctgttccacggcaagatcccctacgtggtggagatggagggcaatgtggatggccacaccttcagcatccgcggcaagggctacggcgatgccagcgtgggcaaggtggatgcccagttcatctgcaccaccggcgatgtgcccgtgccctggagcaccctggtgaccaccctgacctacggcgcccagtgcttcgccaagtacggccccgagctgaaggatttctacaagagctgcatgcccgatggctacgtgcaggagcgcaccatcaccttcgagggcgatggcaatttcaagacccgcgccgaggtgaccttcgagaatggcagcgtgtacaatcgcgtgaagctgaatggccagggcttcaagaaggatggccacgtgctgggcaagaatctggagttcaatttcaccccccactgcctgtacatctggggcgatcaggccaatcacggcctgaagagcgccttcaagatct