Supplemental Information

Supplemental Table 1. Numbers of transcripts differentially regulated in WCR larvae fed on diet supplemented with corn total RNA versus soy total RNA and matched 21 nt long plant-derived siRNAs found in corresponding WCR samples.

Differentially regulated genes, ID#	Annotation	Fold Change	Corn 21mers mapped				Soy 21mers mapped			
			mis0	mis1	mis2	mis3	mis0	mis1	mis2	mis3
TRPT0072526	Aminopeptidase N-like protein	-2.27								
TRPT0065286	Cytochrome P450 9Z5	-1.97								
TRPT0013568	Putative uncharacterized protein	-1.88								
TRPT0101784	C-type lectin-mannose binding	1.51								
TRPT0101783	C-type lectin-mannose binding	1.54								
TRPT0019571	NA	1.70								
TRPT0035292	NA	1.73								1
TRPT0005938	Glycine N-methyltransferase	1.75								
TRPT0068149	RseC_MucC domain containing	1.78								
TRPT0000847	NA	1.80								1
TRPT0005936	Glycine N-methyltransferase	1.82								
TRPT0006922	PBP_GOBP domain containing	1.96								
TRPT0101952	zf-RVT domain containing	2.04			1					
TRPT0068147	NA	2.05								
TRPT0098243	NA	2.13								
TRPT0056635	CDRT4 domain containing	2.33								
TRPT0026715	NA	2.33								
TRPT0053243	NA	2.33								
TRPT0083199	NA	2.33								
TRPT0062667	NA	2.33								
TRPT0069254	Endonuclease-reverse transcriptase	2.33								
TRPT0097612	NA	2.97								

NA- not available

Supplemental Table 2. Number of WCR unigenes (total unigenes numbers=95910) mapped to sRNAs identified in WCR fed on artificial diet supplemented with 50 µg per well of total RNA from corn root, total RNA from soy leaf or fed on intact corn roots.

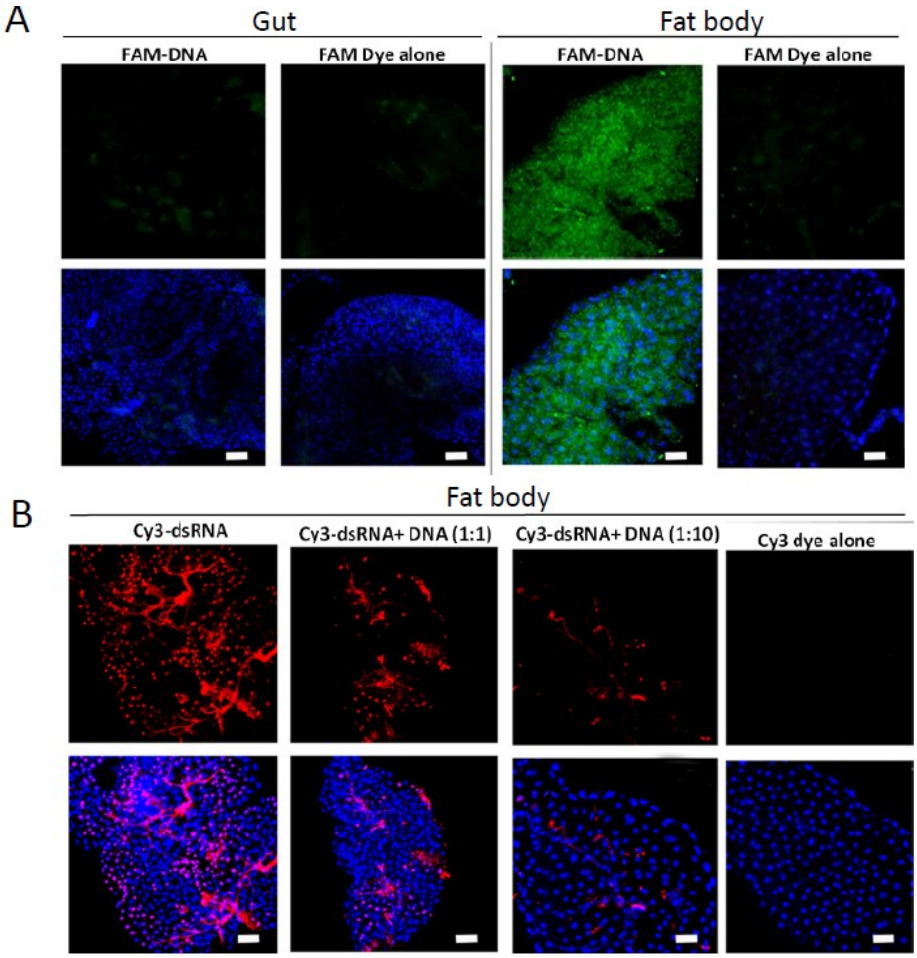
	Corn root RNA	Soy leaf RNA	Corn root
mis0 (perfect match)	149	104	157
mis1	379	407	415
mis2	1561	2197	2613
mis3	4819	7207	8624
Total	**6416**	**8962**	**10673**

Supplemental Table 3. Sequences of dsRNA and constructs used in this study.

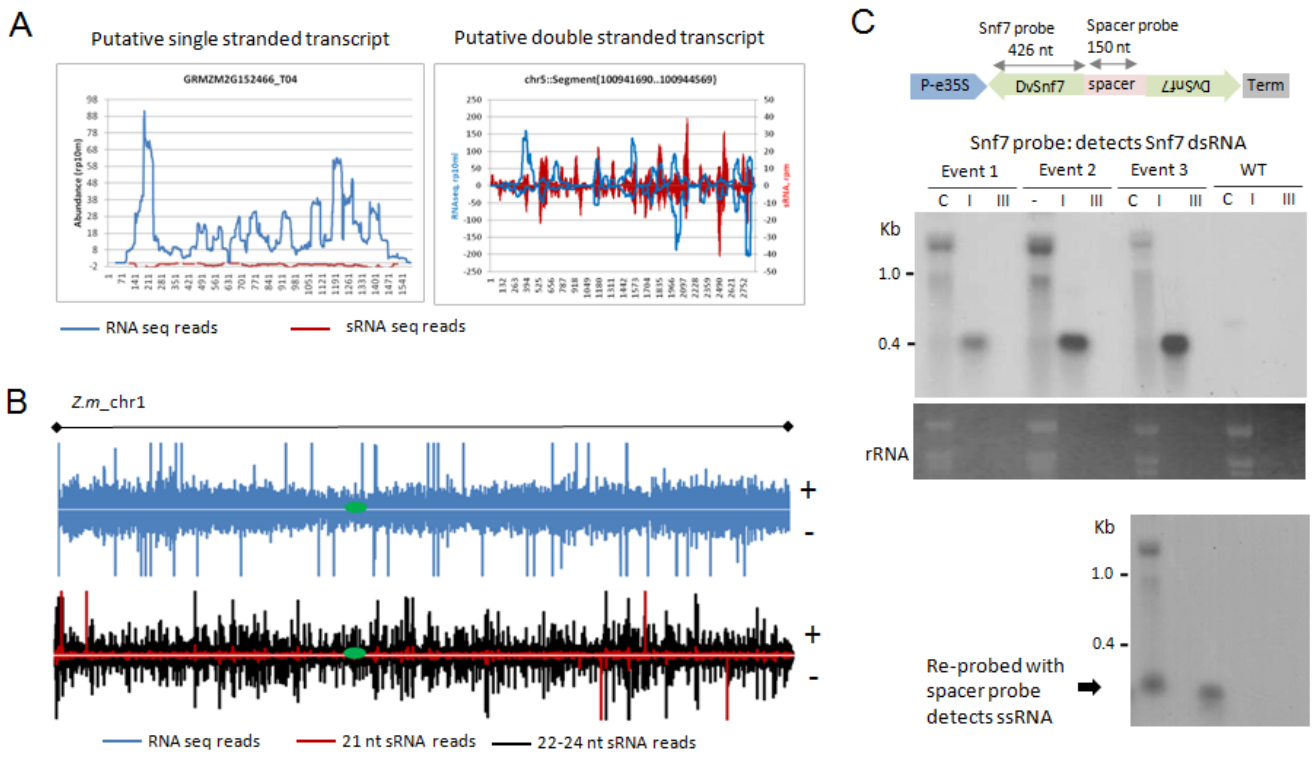
40 mer "neutral" RNA sequence	UAAGUGCAUAAUGUCAAAGUGAAAAAAAAGGUACAUCUAA
60 mer "neutral" RNA sequence	UUUUGUAGAUUAAGUGCAUAAUGUCAAAGUGAAAAAAAAGGUACAUCUAAGAUAGGAAGG
100 mer "neutral" RNA sequence	UCAGUGCCUGAGUGAGAUACUUUUGUAGAUUAAGUGCAUAAUGUCAAAGUGAAAAAAAAG GUACAUCUAAGAUAGGAAGGCGAGAAUGAAUUAUGGAAAU
DvSnf7 27mer embedded in 40 bp "neutral" sequence (*DvSnf7* sequence is highlighted)	UAAGUGCUAGAUGGAACCCUUACAACUAUUGAAAAUCUAA
DvSnf7 27mer embedded in 60 bp "neutral" sequence	UUUUGUAGAUUAAGUGCUAGAUGGAACCCUUACAACUAUUGAAAAUCUAAGAUAGGAAGG
DvSnf7 27mer embedded in 100 bp "neutral" sequence	UCAGUGCCUGAGUGAGAUACUUUUGUAGAUUAAGUGCUAGAUGGAACCCUUACAACUAU UGAAAAUCUAAGAUAGGAAGGCGAGAAUGAAUUAUGGAAAU
240bp non-WCR "neutral" sequence	GCCAAAGAGGAUACUAACCAAAAGCUGGGCGAGUCAGAUGAGGUUCAUAAUGUUACACG ACAGAGAAAGCUCAGUGCCUGAGUGAGAUACUUUUGUAGAUUAAGUGCAUAAUGUCAAA GUGAAAAAAAAGGUACAUCUAAGAUAGGAAGGCGAGAAUGAAUUAUGGAAAUAUCAAUAG GCCCACGUUCCUCCUUGAAGAGCGCUUAUAUAAAUCAUUAUAACAAGAAGGACAAGGCGA AU
Probe for dsRNA detection (dsRNA stability experiment)	AACAGCTATGACCATGATTACGCCAAGCTTTAAGTGCATAATGTCAAAGTG

Green fluorescent protein (GFP) sequence for dsRNA synthesis	GGUCCCAGUUCUUGUUGAAUUAGAUGGCGAUGUUAAUGGGCAAAAAUUCUCUGUCAGUG GAGAGGGUGAAGGUGAUGCAACAUACGGAAAACUUACCCUUAAUUUUAUUUGCACUACU GGGAAGCUACCUGUUCCAUGGCCAACACUUGUCACUACUUUCUCUUAUGGUGUUCAAUG CUUCUCAAGAUACCCAGAUCAUAUGAAACAGCAUGACUUUUUCAAGAGUGCCAUGCCC
Sequences of primers for real-time RT-PCR	*DvSnf7* Forward: CCGACGATCTGGATGACGA *DvSnf7* Reverse: TTACGAGGCCCAGGCTTCC Tubulin Forward: CCAAGAGAGCTTTCGTCCAC Tubulin Reverse: TTCAGCTCCTTCACCCTCAC
Probe sequence for RNase blot (*DvSnf7* segment)	GTCCTGGGGAGGCTATTCAAAAACTCAGAGAGACTGAAGAAATGTTAATAAAAAAACAGGAT TTTTTAGAAAAGAAGATAGAAGAATTTACCATGGTAGCAAAGAAAAATGCGTCGAAAAATAAA AGAGTTGCACTCCAAGCCCTCAAAAAGAAGAAACGATTGGAAAAGACCCAACTACAAATAGA TGGAACCCTTACAACTATTGAAATGCAGAGGGAAGCCCTCGAAGGAGCTAGCACAAATACT GCTGTATTAGATTCTATGAAAAATGCTGCAGATGCCCTTAAGAAAGCTCATAAGAATTTGAAT GTAGATGATGTTCACGATATCATGGATGACATAGCCGAACAACACGACATAGCCAACGAAAT CACAAACGCTATTAGCAATCCTGTCGGATTCACCGACGATCTGGATGACGATG
Probe sequence for RNase blot to detect ssRNA (spacer segment)	AAGTACTGCGATCGCGTTAACGCTTTATCACGATACCTTCTACCACATATCACTAACAACATC AACACTCATCACTCTCGACGACATCCACTCGATCACTACTCTCACACGACCGATTAACTCCT CATCCACGCGGCCGCCTGCAGGAGC
Sequence of *DvSnf7* segment expressed in transgenic corn to produce dsRNA and fed to WCR	CATCGTCATCCAGATCGTCGGTGAATCCGACAGGATTGCTAATAGCGTTTGTGATTTCGTTG GCTATGTCGTGTTGTTCGGCTATGTCATCCATGATATCGTGAACATCATCTACATTCAAATTC TTATGAGCTTTCTTAAGGGCATCTGCAGCATTTTTCATAGAATCTAATACAGCAGTATTTGTG CTAGCTCCTTCGAGGGCTTCCCTCTGCATTTCAATAGTTGTAAGGGTTCCATCTATTTGTAG TTGGGTCTTTTCCAATCGTTTCTTCTTTTTGAGGGCTTGGAGTGCAACTCTTTTATTTTTCGA CGCATTTTTCTTTGCTACCATGGTAAATTCTTCTATCTTCTTTTCTAAAAAATCCTGTTTTTTTA TTAACATTTCTTCAGTCTCTCTGAGTTTTTGAATAGCCTCCCCAGGAC

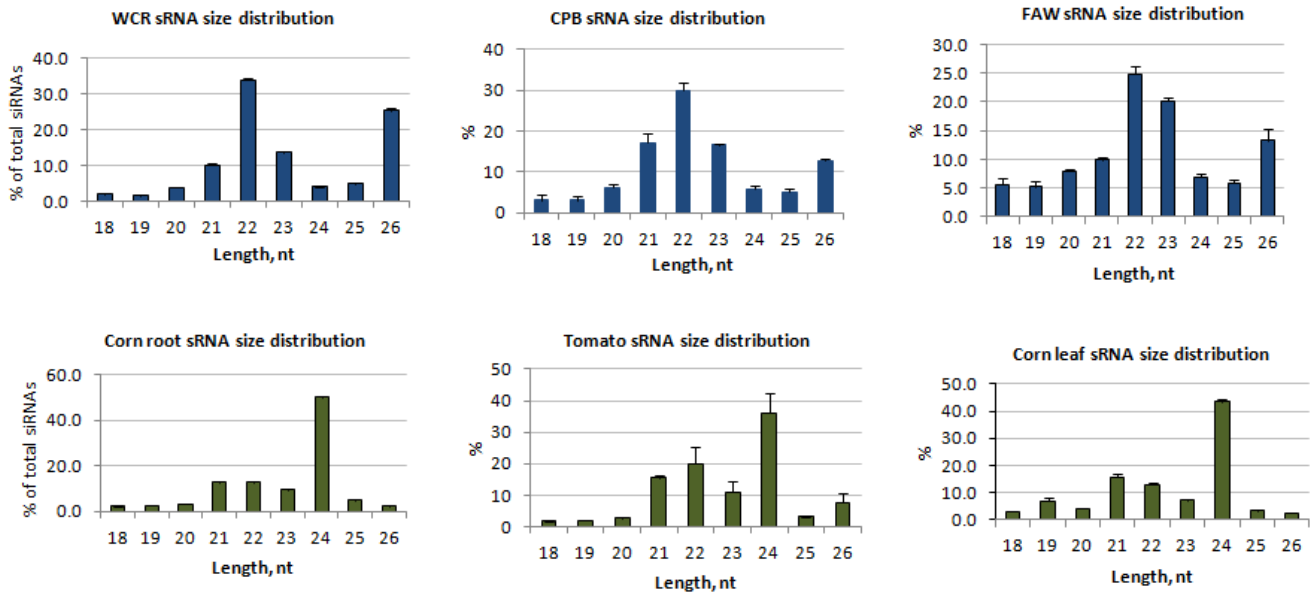
DvSnf7-240 bp dsRNA used for uptake study	GCAAAGAAAAAUGCGUCGAAAAAUAAAAGAGUUGCACUCCAAGCCCUCAAAAAGAAGAAA CGAUUGGAAAAGACCCAACUACAAAUAGAUGGAACCCUUACAACUAUUGAAAUGCAGAGG GAAGCCCUCGAAGGAGCUAGCACAAAUACUGCUGUAUUAGAUUCUAUGAAAAAUGCUGCA GAUGCCCUUAAGAAAGCUCAUAAGAAUUUGAAUGUAGAUGAUGUUCACGAUAUCAUGGAU
DvSnf7-21 bp siRNA used for uptake study, [U] - overhang	GAUGGAACCCUUACAACUA[U][U]
"Neutral" sequence (402 bp) used for producing dsRNA in transgenic corn (one strand is shown)	CCAAGATCTCACCAGCTATAAATCGAGTGAGTGCGAACGCCGGCCTCACTCTTGATCGGAG CGAGTAGCTAGGCAGCATGACGCATGAGATGAACGGGTCCCTGAATCTCTGTCAGCATATA TACACATACACGGAGGTAGCCGCCGCCGAATTCGTCGTGCTCCCATGTCCATCCGATTGGT CGTCATCAAGGCTGTCTCCAGGCTTGCTATAGCTGGTCCATGGCACCATACATGTAAGCAC GCACACAGGCACACACACACACGCACGCAATGATCTACGTATCTAGCAGCAGCTTATCATG TCGTCATCATGCATGCATGGCCGACGGAGGTCGTCATCTTATCTGGGAGCGTGTGTGTCTT GGCAATGGGAAGCTGCATGCGCCTCTCGGCCGGACG



Supplemental Figure 1. *(A)* Determination of uptake of labeled dsDNA by WCR tissues. Fat bodies and midgut tissues were dissected from second instar WCR (n=5) and exposed to labeled dsDNA for 15 hours *in vitro*. Snf7 dsDNA (240 bp) was labeled with FAM-siRNA labeling kit (Ambion). Controls included FAM dye (panel A) alone and medium alone (data not shown). Excitations of 488 nm (FAM) and 395 nm (DAPI) were used. Upper images capture green signal from applied dsDNA uptake, while lower images are merged images showing uptake of dsDNA and blue fluorescence from DAPI nuclear DNA staining. Scale Bar: 50 µm. *(B) In vitro* competition of dsRNA:dsDNA uptake by WCR fat body. Fat bodies were dissected from second instar WCR (n=5) and exposed to Cy3-labeled Snf7 dsRNA or mixture of Cy3–labeled dsRNA and unlabeled Snf7 dsDNA (240 bp) in the ratio of 1:1 and 1:10. Snf7 dsRNA (240 bp) was labeled with Cy3-siRNA labeling kit (Ambion). Unlabeled Snf7 dsDNA was synthesized from plasmid clone of Snf7 by PCR reaction using gene specific primers. Controls included Cy3 dye alone (panel B) and medium alone (data not shown). Excitations of 543 nm (Cy3) and 395 nm (DAPI) were used. Upper images capture red dsRNA signal, while lower images are merged images showing uptake of dsRNA and blue fluorescence from DAPI nuclear DNA staining. Scale Bar: 50 µm. The tissues were processed and observed under confocal microscope as described in Bolognesi et al. (2012).

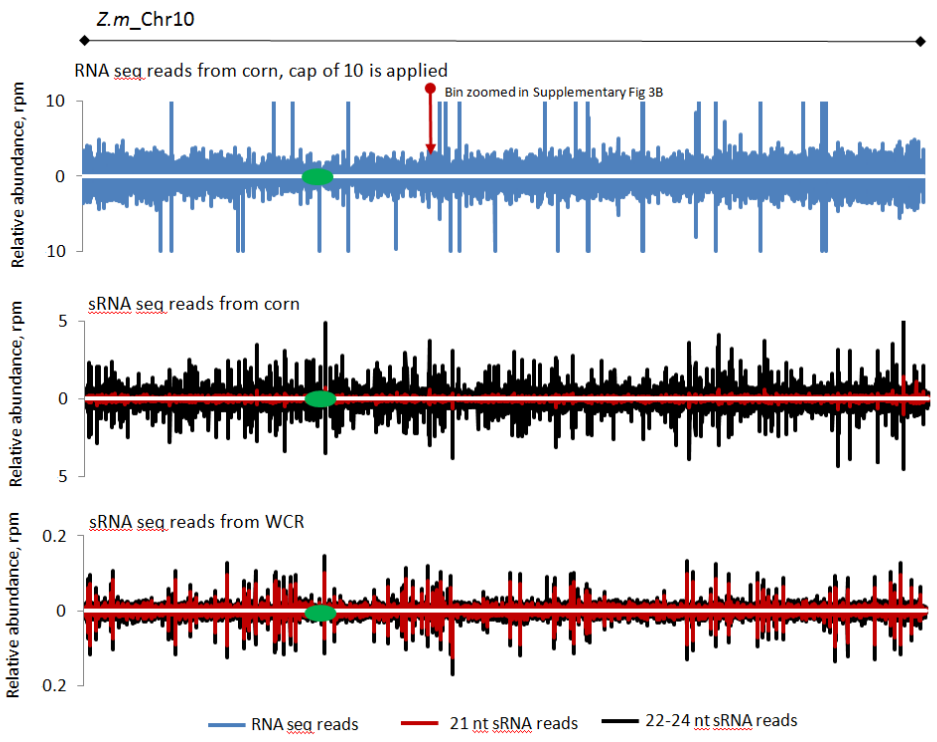


Supplemental Figure 2. Evidence for long dsRNA accumulation in corn. (*A*) Example shown from mapping of strand-specific RNA-seq and sRNA reads identifying putative dsRNA-producing loci in the corn genome with distinct profiles from single strand-producing loci. (*B*) Genome wide mapping of strand-specific RNA-seq and sRNA sequence reads to the corn genome revealed widespread distribution and overlap between RNA-seq and siRNA hotspot clusters mapped to both strands of the corn genome. Corn chromosome one is shown as an example. Centromere location is shown as a green oval. (*C*) Northern blot analysis of long dsRNAs transcribed from an inverted repeat cassette in corn with sequence derived from the WCR *DvSnf7* gene revealed significant accumulation of intact long dsRNA. RNA was resolved on a denaturing gel and blotted and probed using the probe in Table S3. C: untreated, I: RNase I-treated, III: RNase III-treated total RNA from transgenic lines 1, 2, 3 and wild type (WT) corn samples.

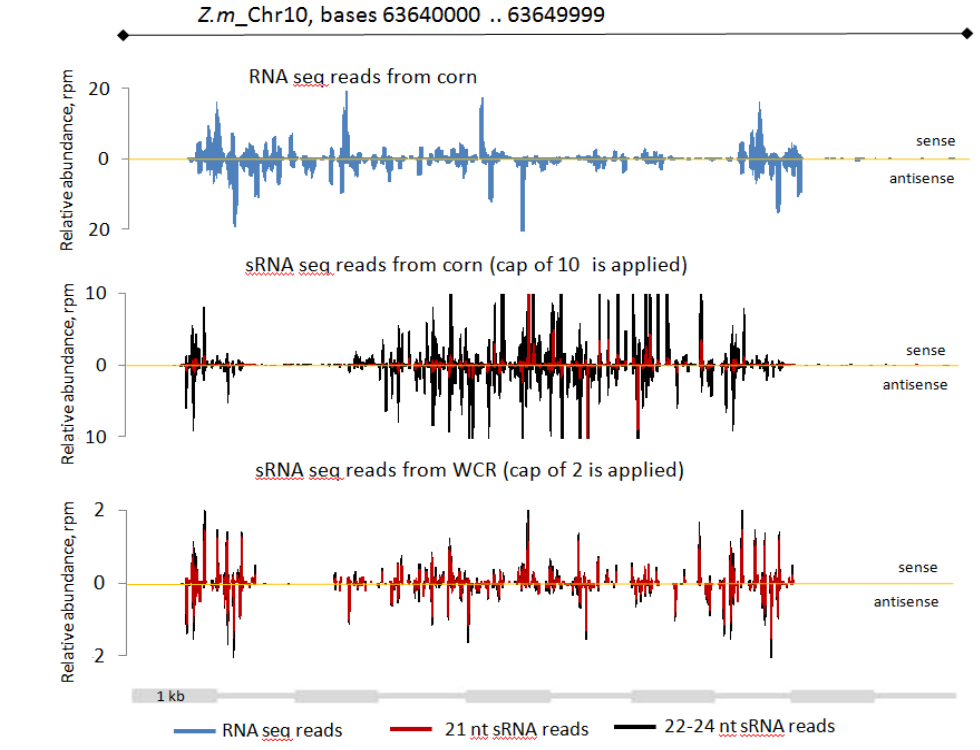


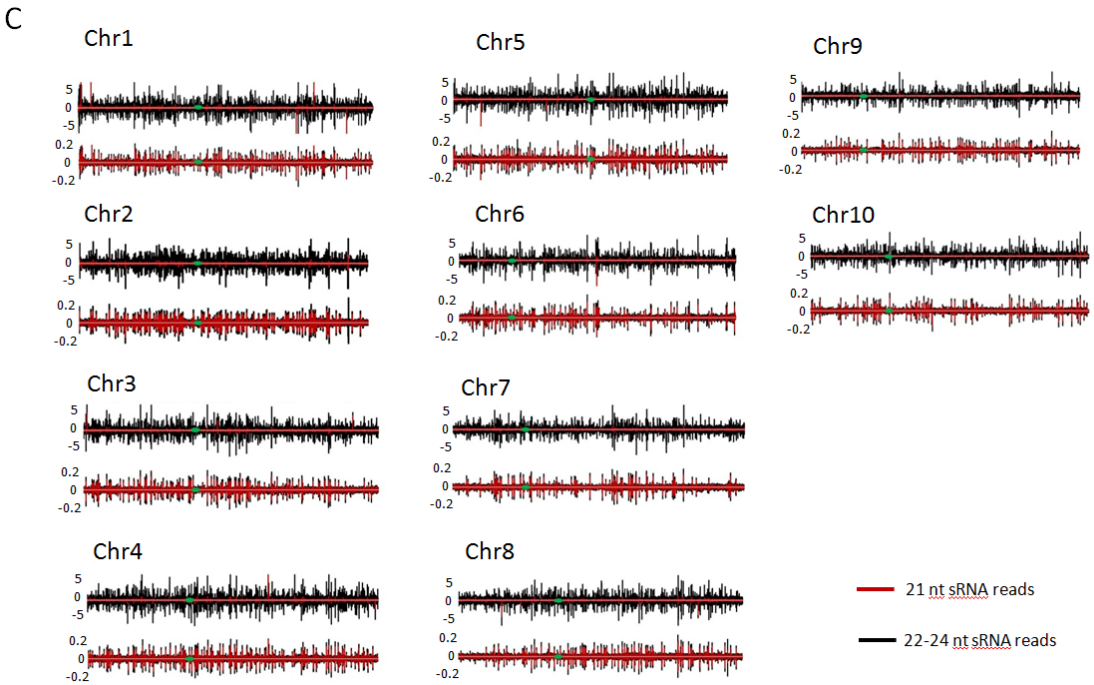
Supplemental Figure 3. sRNA length distribution and abundance in insect and host plants food sources. Percent of total graphed vs. sRNA length.

A

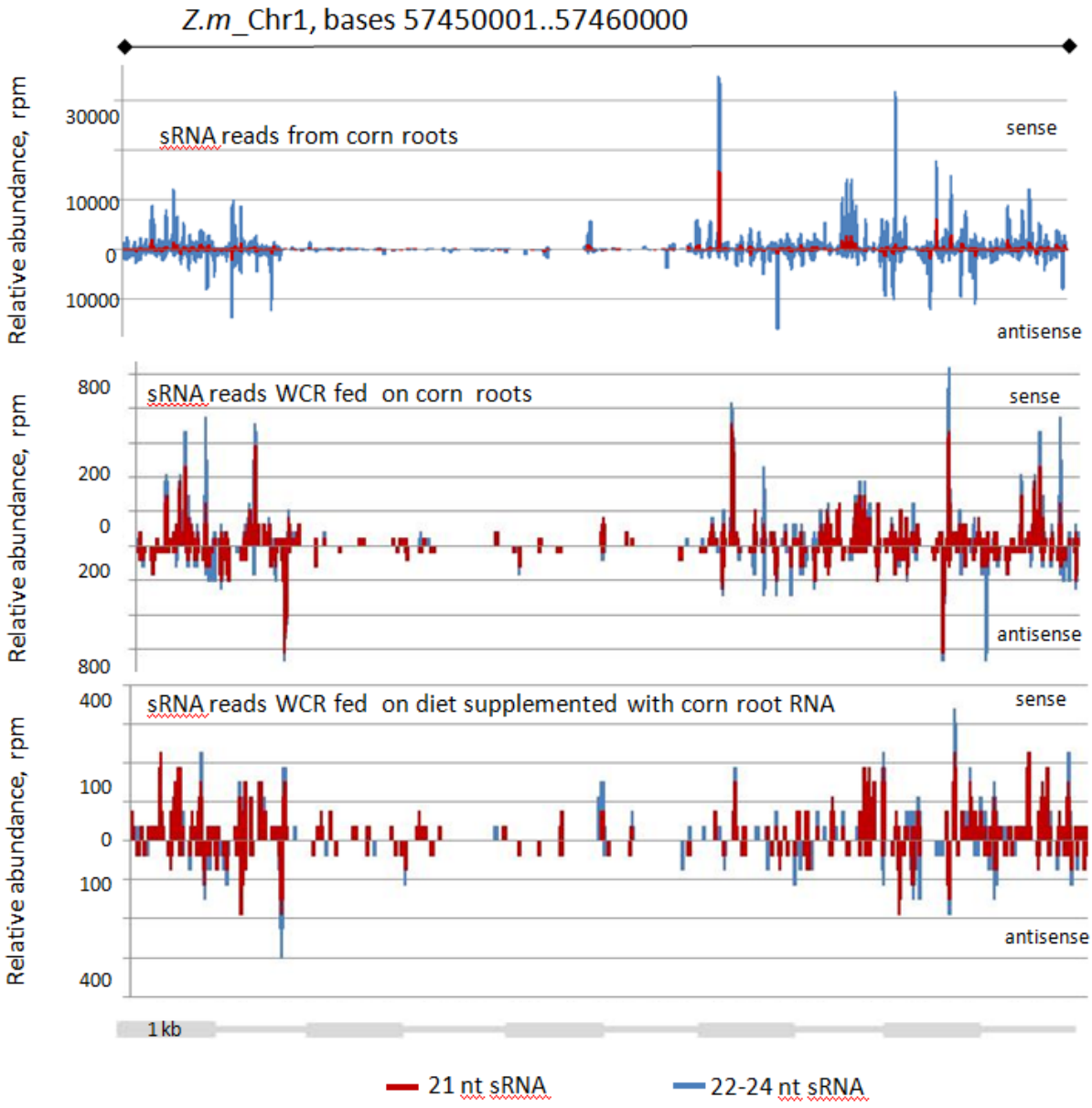

Z.m_Chr10
RNA seq reads from corn, cap of 10 is applied
Bin zoomed in Supplementary Fig 3B
Relative abundance, rpm
10
0
10
sRNA seq reads from corn
5
0
5
sRNA seq reads from WCR
0.2
0
0.2
RNA seq reads
21 nt sRNA reads
22-24 nt sRNA reads

B

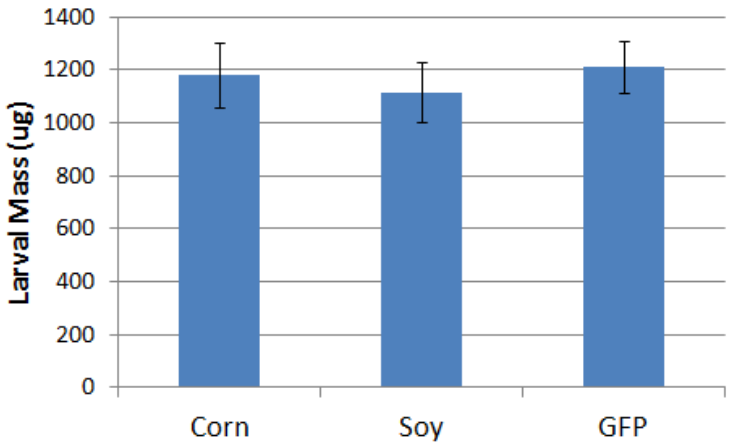

Z.m_Chr10, bases 63640000 .. 63649999
RNA seq reads from corn
sense
antisense
sRNA seq reads from corn (cap of 10 is applied)
sRNA seq reads from WCR (cap of 2 is applied)
Relative abundance, rpm
1 kb
RNA seq reads
21 nt sRNA reads
22-24 nt sRNA reads



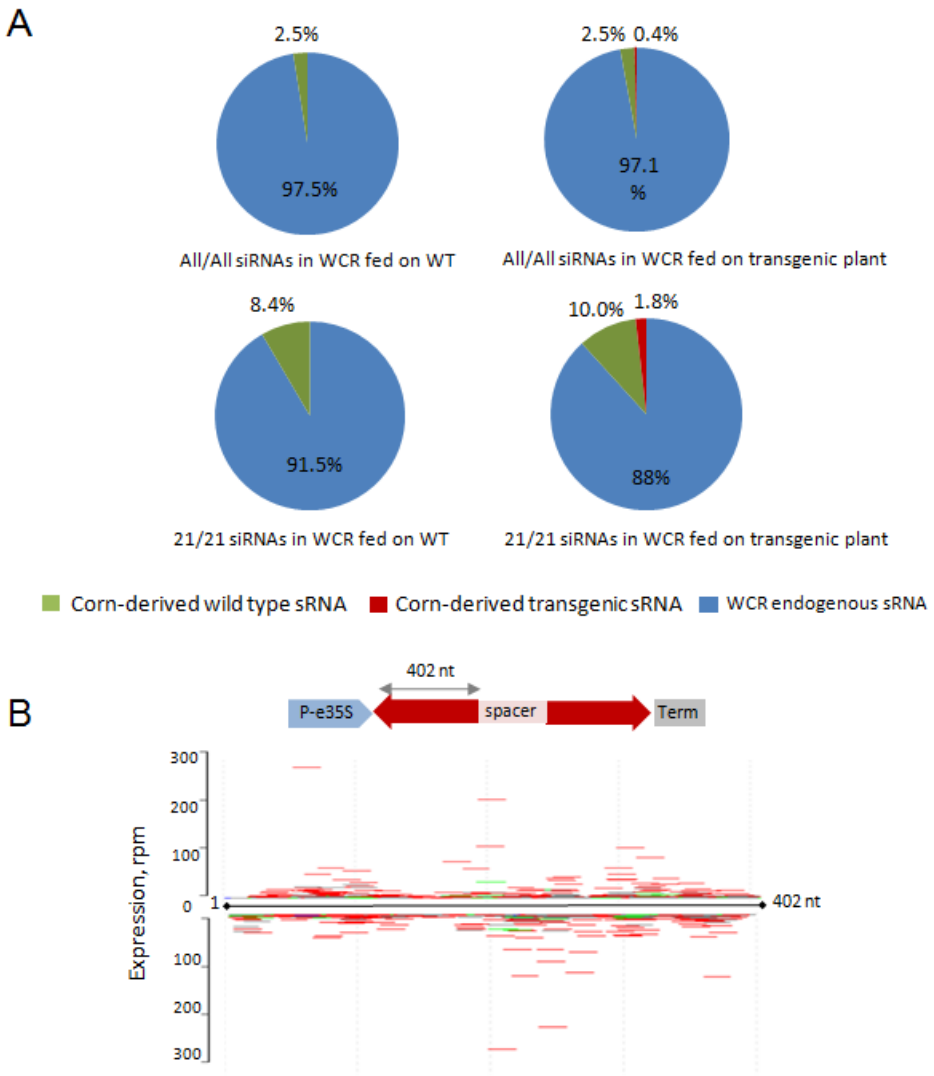
Supplemental Figure 4. (*A*) Genome wide mapping to corn genome of corn strand-specific RNA-seq and sRNA sequence reads. sRNAs from WCR larvae fed on corn roots were similarly mapped. The chromosome was binned into 10 kb segments where abundance of each base in the bin is averaged. Public genome assembly v2 (www.maizegdb.org/) was used to determine centromere position (green oval). Corn chromosome 10 is shown as an example. (*B*) An expanded individual 10kb region of corn chromosome 10. (*C*) Genome wide mapping to corn genome of small RNA sequence reads from corn roots (upper map of each chromosome display) and from carcasses of WCR larvae fed on corn roots (lower map of each chromosome display). All ten corn chromosomes are shown. The Y-axis shows realtive abundance in rpm (reads per million) for corn genome -mappable RNA seq or sRNAs, respectively. In Figure 3A and C the abundance is also normalized to per base for each window.



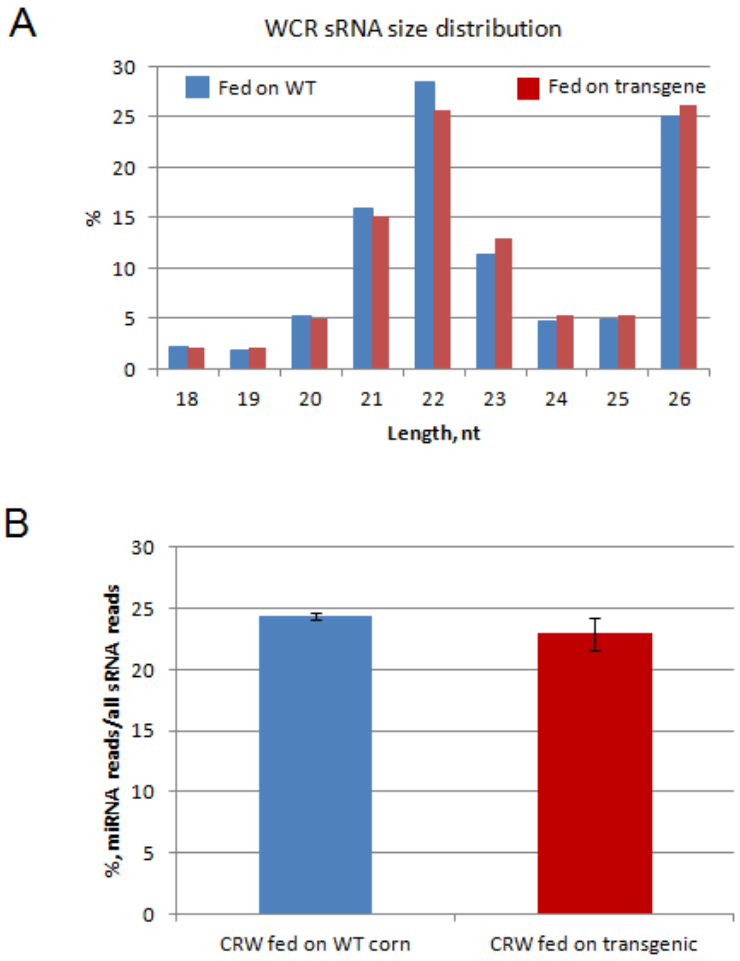
Supplemental Figure 5. siRNAs from corn and WCR fed on either corn or diet mapped to corn chromosome. Corn siRNAs from corn roots, corn-derived siRNAs detected in WCR larvae fed on corn roots, and corn-derived siRNAs from WCR larvae fed on diet supplemented with corn root total RNA were mapped to a10 kb region of corn chromosome 1: 57450001..57460000. Relative abundance: rpm (reads per million) of corn genome mappable sRNAs.



Supplemental Figure 6. Effect of dsRNA on WCR larval development. WCR larvae were fed for 4 days on artificial diet supplemented with 50 µg of total RNA from corn roots, soy leaves or in vitro synthesized control dsRNA (GFP sequence). Error bars show standard deviation.



Supplemental Figure 7. Abundance of sRNAs in WCR larvae fed on wild type or transgenic corn expressing long dsRNA. (*A*) Percentage of non transgenic and transgenic plant-derived siRNAs relative to insect sRNAs for sRNAs (18-26nt) vs. 21nt only. (*B*) Mapping of plant-derived siRNAs identified in WCR fed on corn expressing transgenic long dsRNA to sequence used for expressing dsRNA.



Supplemental Figure 8. Comparison of WCR sRNA characteristics in larvae fed on wild type (WT) or transgenic corn roots. (*A*) sRNA size distribution in WCR fed on WT or transgenic corn roots for 4 days. (*B*) miRNA abundance relative to all siRNA reads in WCR fed on WT or transgenic corn roots for 4 days. Error bars show standard deviation.