New Phytologist Supporting Information

Article title: The *Parastagonospora nodorum* necrotrophic effector SnTox5 targets the wheat gene *Snn5* and facilitates entry into the leaf mesophyll
Authors: Gayan K. Kariyawasam, Jonathan K. Richards, Nathan A. Wyatt, Katherine L.D. Running, Steven S. Xu; Zhaohui Liu, Pawel Borowicz, Justin D. Faris and Timothy L. Friesen
Article acceptance date: 27 June 2021

The following Supporting Information is available for this article:

**Fig. S1** Nucleotide sequence of *SnTox5* and its promoter region

**Fig. S2** Detailed secondary structure prediction of the mature SnTox5

**Fig. S3** PCR confirmation of *SnTox5* gene deletion in Sn2kΔTox5 strains

**Fig. S4** Pairwise alignment of 20 isoforms of SnTox5

**Fig. S5** Laser confocal microscopy of Sn79-1087 and Sn79+Tox5 on LP29 and LP29Asnn5

**Fig. S6** Volume analysis of Sn2000 and Sn2kΔTox5-15 on LP29 and LP29Asnn5

**Table S1** Primers used in this study

**Table S2** Phenotypic data used in GWAS analysis

**Table S3** Temporal expression of *SnTox5*

**Methods S1** Disease phenotyping

**Methods S2** Whole genome sequencing and variant identification

**Methods S3** Genome-wide association mapping

**Methods S4** QTL analysis of the LP749 population

**Methods S5** Development of construct of *SnTox5* using Gateway cloning system

**Methods S6** Inoculation and infiltration of gain-of-function transformants

**Methods S7** Extraction of *SnTox5* sequence for population genetics and haplotype analysis

**Methods S8** Development of *Snn5* mutants of LP29
Fig. S1 Nucleotide sequence of the SnTox5 gene of Sn2000 used to transform Sn79-1087 to create the gain of function transformants Sn79+Tox5-3 and Sn79+Tox5-4 and the resulting amino acid sequence of SnTox5. Yellow color indicates the putative TATAA box 171 bp upstream of the start codon. Purple, red, and green colors indicate the signal peptide, the pro-domain and the putative Kex2 site of the protein.
**Fig. S2** Detailed secondary structure prediction of the mature SnTox5 by Phyre\(^2\). Blue arrows represent β-strands whereas green helix represents the α-helix. The α-helix was only predicted at low confidence by Phyre2 and was not predicted by Chimera (See Fig 2). The colors represent the confidence in prediction along the sequence. Question marks (?) represent the disordered regions of the protein.
**Fig. S3** PCR confirmation of *SnTox5* gene deletion in Sn2kΔTox5 strains. M: Marker, 1: Sn2kΔTox5-8, 2: Sn2kΔTox5-9, 3: Sn2kΔTox5-10, 4: Sn2kΔTox5-15, 5: Sn2kΔTox5-17, 6: Sn2000 (positive control), 7: H₂O (negative control).
Fig. S4  Pairwise alignment of 20 isoforms using isoform 1 as the reference.

Isoform 1: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 2: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 3: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 4: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 5: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 6: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 7: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 8: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 9: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 10: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 11: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 12: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 13: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 14: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 15: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 16: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 17: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 18: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 19: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
Isoform 20: MFLKSLFIV QUAXTYPQ LEQESLTSQ TXTNATAPLQ DXEVEGEGI RTQQKASQ TPQMEQIQE KQTVQTQP QTVSTNW 120
**Fig. S5** Laser confocal microscopy of red fluorescent protein (RFP) tagged Sn79-1087 and Sn79+Tox5 on LP29 and LP29Δsnn5 at 120 hpi. Micrographs of RFP tagged Sn79 (-SnTox5) and Sn79+Tox5 (+SnTox5) inoculated on LP29 (Snn5), and LP29Δsnn5 (snn5) at 120 hpi. I). Sn79-1087 was able to colonize the epidermis but not the mesophyll of LP29. II). Sn2kΔTox5 formed a penetration structure on LP29Δsnn5. However, Sn79-1087 was not able to penetrate the epidermis of LP29Δsnn5 since the interaction lacked SnTox5 and Snn5. III). Sn79+Tox5 was able to penetrate and colonize both epidermis and mesophyll tissue of LP29 since interaction was able to establish SnTox5-Snn5 interaction. IV). Sn79+Tox5 was able to colonize the first layer of mesophyll of LP29Δsnn5, but failed to cause PCD due to the lack of Snn5. G=Germ tube; S= Spore; PS= Penetration structure; CE= Colonization of epidermis; CM= Colonization of mesophyll.
**Fig. S6** Volume of the fungus calculated through laser confocal microscopy at 120 hours post inoculation (hpi) for the inoculations of Sn2000 (+SnTox5) and Sn2kΔTox5-15 (-SnTox5) on LP29 (Snn5) and LP29Δsnn5 (snn5). The x-axis represents the strain-host combination and the y-axis represents the volume of the fungus at 120 (hpi) in µm³. Error bars represent the standard error of the mean from three replications. The fungal volume of Sn2000 in LP29 was significantly higher than the rest of the combinations. Though Sn2000 was not able to completely colonize the mesophyll of LP29Δsnn5 due to the lack of PCD, the volume of the Sn2000 was significantly higher than that of Sn2kΔTox5-15 on both LP29 and LP29Δsnn5 showing further evidence for the role of the SnTox5 in progression of the fungal mycelia into the mesophyll layer.
| ID                  | Sequence*                                                            |
|---------------------|---------------------------------------------------------------------|
| Tox5HygDonor F1*    | ATAGTCCATTGTCACTGGACAGAGGTAGCAGGATTACAAATTTGAACACGAGGACAGG          |
| Tox5HygDonor R1     | AACGGAAATTGGCAGGTGTAGAGCGCTGTTCCTTTAGGGAGCAGGAAACAGCTATGAC          |
| Tox5_sgRNA          | TTCTAATACGACTACTATAGCTGTTCCTTTAGGGACGTCTGTTTTAGAGCTAG              |
| SnTox5_pENTR_F1_bae | CACCATGGATGCGGAACATTACGGGAGAGCAA                                   |
| SnTox5_pENTR_R1     | CGTGTTCTGGGAACATCCCTGTT                                           |
| SnTox5_DONOR_F      | GGGGACAGTTGTTGACAAAAAGCAGGCTTTGTGCGCTCTCATCAAATCG                 |
| SnTox5_DONOR_R      | GGGGACACCTTTGTACAGGAAGCTGGGTGCCGTCTTAAGCTGGGACATCC                 |
| Tox5_Seq_F          | ACACCAACCACTTTGCGCTTAAGCA                                       |
| M13F                | GACGTTGAAACGACGAGG                                               |
| M13R                | CACAGGAAACAGCTATGACATGA                                         |
| HY                  | GAATGCCTCAGCTCGAGA                                               |
| YG                  | CGTTGGAACACCTGCTGAA                                              |
| PnActin_F           | GCGGTTGGCAATCCACGTCCA                                         |
| PnActin_R           | TGCGATGATCCTGACCTTCATGAC                                         |
| SnTox5_qPCR_F       | TGTAATGTCAACAAAGGCAATCTAC                                       |
| SnTox5_qPCR_R       | AGGCAAAAGTGTGGCAATGAA                                            |

*Bolded sequence indicates the 40 bp region homologous to the flanking region of protospacer adjacent motif that used to disrupt SnTox5
## Table S2 Phenotypic data used in GWAS analysis

| Isolate   | Replicate 1* | Replicate 2* | Replicate 3* | Average* disease reaction |
|-----------|--------------|--------------|--------------|--------------------------|
| 98-13025-2 | 1            | 0            | 0.5          | 0.50                     |
| 98-13042-1 | 2            | 4            | 2.5          | 2.83                     |
| 98-13050-1 | 0            | 0.5          | 1.5          | 0.67                     |
| 98-13063-1 | 4.5          | 2.5          | 3.5          | 3.50                     |
| 98-13066   | 3            | 4            | 2.5          | 3.17                     |
| 98-13082   | 4            | 4            | 3            | 3.67                     |
| 98-13091-2 | 3.5          | 3            | 2            | 2.83                     |
| AR1-1      | 3            | 4.5          | 3.5          | 3.67                     |
| AR2-1      | 3.5          | 3            | 2.5          | 3.00                     |
| AR3-1      | 4            | 4.5          | 3            | 3.83                     |
| AR4-1      | 4            | 4.5          | 2            | 3.50                     |
| AR5-1      | 0            | 0            | 0.5          | 0.17                     |
| AR6-1      | 2.5          | 2.5          | 1.5          | 2.17                     |
| BBC03 Sn-1 | 0            | 0            | 0.5          | 0.17                     |
| BBC03 Sn-3 | 0.5          | 1.5          | 0            | 0.67                     |
| BBC03 Sn-5 | 4            | 3.5          | 3.5          | 3.67                     |
| BBC04 Sn-1 | 1            | 2            | 3.5          | 2.17                     |
| BBC04 Sn-2 | 0.5          | 1.5          | 0            | 0.67                     |
| BBC04 Sn-5 | .            | .            | .            | .                        |
| BBC04 Sn-6 | 0            | 1.5          | 2            | 1.17                     |
| FgoG10 Sn-1| 0.5          | 0.5          | 2            | 1.00                     |
| FgoN10 Sn-2| 1.5          | 3.5          | 2            | 2.33                     |
| FgoN10 Sn-3| 4            | 4            | 2            | 3.00                     |
| FgoN10 Sn-4| 2.5          | 2            | 1.5          | 2.00                     |
| GA9-1      | 1            | 4            | 2.5          | 2.50                     |
| GA9-2      | 4            | 4            | 4            | 4.00                     |
| GA9-3      | 3            | 3            | 2            | 2.67                     |
| GA9-4      | 3.5          | 3            | 2.5          | 3.00                     |
| GA9-5      | 1.5          | 1            | 0.5          | 1.00                     |
| LDN03 Sn-1 | 2.5          | 3.5          | 4            | 3.33                     |
| LDN03 Sn-10| 1.5          | 3.5          | 2.5          | 2.50                     |
| LDN03 Sn-11| 1            | 2.5          | 1.5          | 1.67                     |
| LDN03 Sn-2 | 4.5          | 2            | 4.5          | 3.67                     |
| LDN03 Sn-3 | .            | .            | 3.5          | 4            | 3.75                     |
| LDN03 Sn-4 | .            | .            | 3.5          | 2            | 2.75                     |
| LDN03 Sn-5 | 0.5          | 1.5          | 0.5          | 0.83                     |
| Label | Value1 | Value2 | Value3 | Value4 |
|-------|--------|--------|--------|--------|
| LDN03 Sn-6 | 1.5 | 1.5 | 2 | 1.67 |
| LDN03 Sn-7 | 4 | 4.5 | 4.5 | 4.33 |
| LDN03 Sn-8 | 2 | 2 | 2 | 2.00 |
| LDN03 Sn-9 | 3.5 | 4 | 1 | 2.25 |
| LDN05 SN-2 | 1.5 | 2.5 | 2 | 2.00 |
| LDN05 Sn-4 | 3 | 0.5 | 0 | 1.17 |
| LDN05 Sn-5 | 0.5 | 0 | 0.5 | 0.50 |
| LDN07 Sn-1 | 0 | 2 | 0.5 | 0.83 |
| LDN07 Sn-2 | 0 | 2.5 | 3 | 1.83 |
| LDN07 Sn-3 | 0 | 0 | 0 | 0.00 |
| LDN07 Sn-4 | 0 | 1 | 0 | 0.33 |
| LDN07 Sn-5 | 0 | 1.5 | 2 | 1.17 |
| LDN08 Sn-1 | 0 | 0 | 0.5 | 0.17 |
| LDN08 Sn-2 | 0 | 1.5 | 0.5 | 0.67 |
| LDN08 Sn-3 | 2 | 1.5 | 2 | 1.83 |
| LDN08 Sn-4 | 2 | 0 | 2 | 1.33 |
| LDN08 Sn-5 | 0 | 3 | 1.5 | 1.50 |
| MD4-1 | 0.5 | 0.5 | 0.5 | 0.50 |
| MD4-2 | 0 | 2.5 | 0 | 1.25 |
| MD4-3 | 2 | 0 | 2 | 1.33 |
| MN-2 | 0 | 0 | 0.5 | 0.17 |
| MN-3 | 2.5 | 1.5 | 1.5 | 1.83 |
| MN-4 | 0 | 1.5 | 0.5 | 0.67 |
| MN-5 | 0 | 0 | 1.5 | 0.50 |
| MN-6 | 1.5 | 0 | 0 | 0.50 |
| MN-7 | 1.5 | 0 | 1.5 | 1.00 |
| MN-8 | 0 | 0 | 2 | 0.67 |
| NC 7-1 | 0.5 | 1.5 | 0.5 | 0.83 |
| NC 8-11 | 2.5 | 2.5 | 2.5 | 2.50 |
| NC 8-7 | 2 | 4 | 2.5 | 2.83 |
| NC 9-12 | 2 | 3 | 3.5 | 2.83 |
| NC 9-5 | 3 | 4.5 | 4 | 3.83 |
| NC8-3 | 0 | 3 | 3 | 3.00 |
| NC8-4 | 2 | 3 | 3.5 | 2.83 |
| NC8-6 | 2.5 | 2 | 2 | 2.17 |
| NC8-8 | 4 | 3 | 4 | 3.67 |
| NDJ16 Sn-1 | 2 | 1.5 | 1.5 | 1.67 |
| NDJ16 Sn-10 | 3.5 | 2.5 | 1.5 | 2.50 |
| NDJ16 Sn-11 | 3.5 | 1 | 1.5 | 2.00 |
| NDJ16 Sn-12 | 2 | 2 | 3.5 | 2.50 |
| NDJ16 Sn-13 | 2.5 | 0 | 4 | 2.17 |
| NDJ16 Sn-14 | 1.5 | 3 | 1.5 | 2.00 |
| NDJ16 Sn-15 | 0 | 0 | 0 | 0.00 |
| Sample | Value1 | Value2 | Value3 | Value4 |
|--------|--------|--------|--------|--------|
| NDJ16Sn-16 | 0 | 1.5 | 0 | 0.50 |
| NDJ16Sn-18 | 2.5 | 2.5 | 3 | 2.67 |
| NDJ16Sn-17 | 1 | 0.5 | 1.5 | 1.00 |
| NDJ16Sn-19 | 2 | 0.5 | 3 | 1.83 |
| NDJ16Sn-2 | 0.5 | 3 | 3 | 2.17 |
| NDJ16Sn-20 | 0 | 0 | 2 | 0.67 |
| NDJ16Sn-21 | 2 | 0 | 1.5 | 1.17 |
| NDJ16Sn-22 | 4 | 0.5 | 2.5 | 2.33 |
| NDJ16Sn-23 | 4 | 2 | 0.5 | 2.17 |
| NDJ16Sn-24 | 2 | 1 | 2 | 1.67 |
| NDJ16Sn-25 | 2.5 | 1 | 0.5 | 1.33 |
| NDJ16Sn-3 | 1.5 | 0.5 | 2.5 | 1.50 |
| NDJ16Sn-4 | 4 | 2.5 | 3 | 3.17 |
| NDJ16Sn-5 | 0.5 | 0 | 0 | 0.17 |
| NDJ16Sn-6 | 0.5 | 2.5 | 3.5 | 2.17 |
| NDJ16Sn-7 | 1.5 | 1 | 1 | 1.17 |
| NDJ16Sn-8 | 3.5 | 3 | 2.5 | 3.00 |
| NDJ16Sn-9 | 1.5 | 1.5 | 1.5 | 1.50 |
| NDM16 Sn-1 | 1 | 2.5 | 1.50 |
| NDM16 Sn-10 | 3.5 | 2.5 | 2.5 | 2.83 |
| NDM16 Sn-2 | 3 | 3.5 | 3.5 | 3.33 |
| NDM16 Sn-3 | 1.5 | 3.5 | 0 | 1.67 |
| NDM16 Sn-4 | 2.5 | 1.5 | 3 | 2.33 |
| NDM16 Sn-5 | 4 | 3.5 | 4 | 3.83 |
| NDM16 Sn-6 | 1 | 3 | 0.5 | 1.50 |
| NDM16 Sn-7 | 1.5 | 3 | 2.5 | 2.33 |
| NDM16 Sn-8 | 1 | 0.5 | 1 | 0.83 |
| NDM16 Sn-9 | 1 | 1.5 | 1.5 | 1.33 |
| NDP16 SN-1 | 1.5 | 1 | 1.5 | 1.33 |
| NDP16 SN-10 | 3 | 1.5 | 3 | 2.50 |
| NDP16 Sn-11 | 3 | 2.5 | 2.75 |
| NDP16 Sn-12 | 4 | 3.5 | 3 | 3.50 |
| NDP16 SN-2 | 0.5 | 0 | 0 | 0.17 |
| NDP16 SN-3 | 0 | 0 | 0 | 0.00 |
| NDP16 SN-4 | 3.5 | 3.5 | 4 | 3.67 |
| NDP16 SN-5 | 3 | 1.5 | 0.5 | 1.67 |
| NDP16 SN-6 | 0.5 | 2 | 1.5 | 1.33 |
| NDP16 SN-7 | 3.5 | 3 | 2.5 | 3.00 |
| OH03 Sn-1051 | 3 | 4 | 4 | 3.67 |
| OH03 Sn-1180 | 3 | 2 | 4.5 | 3.17 |
| OH03 Sn-123 | 3.5 | 3.5 | 3.5 | 3.50 |
| OH03 Sn-1354 | 2.5 | 3.5 | 4.5 | 3.50 |
| OH03 Sn-14 | 4 | 2.5 | 3.5 | 3.33 |
| Material | ID   | 1   | 2   | 3   |
|----------|------|-----|-----|-----|
| OH03 Sn-1501 | 3 | 3.5 | 3.25 |
| OH03 Sn-1553 | 2.5 | 1.5 | 2.5 | 2.17 |
| OH03 Sn-39 | 3 | 4.5 | 3.5 | 3.67 |
| OH03 Sn-601 | 2.5 | 2.5 | 4.5 | 3.17 |
| OH03 Sn-61 | 0.5 | 2 | 1.25 |
| OH03 Sn-65 | 3 | 2.5 | 4.5 | 3.33 |
| OH03 Sn-69 | 4 | 4 | 3 | 3.67 |
| OH03 Sn-8 | 2.5 | 4 | 3.5 | 3.33 |
| OH03 Sn-801 | 0.5 | 0.5 | 0 | 0.33 |
| OH03 Sn-84 | 3.5 | 3 | 4.5 | 3.67 |
| OH03 Sn-850 | 4.5 | 2 | 2 | 2.83 |
| OKG16-1 | 3 | 2.5 | 3 | 2.83 |
| OKG16-10 | 3 | 3 | 2 | 2.67 |
| OKG16-11 | 3 | 3.5 | 3 | 3.17 |
| OKG16-12 | 3.5 | 3.5 | 3.5 | 3.50 |
| OKG16-13 | 2 | 2 | 1.5 | 1.83 |
| OKG16-14 | 2 | 1.5 | 1.5 | 1.67 |
| OKG16-15 | 1.5 | 3.5 | 2 | 2.33 |
| OKG16-16 | 2 | 1.5 | 3 | 2.17 |
| OKG16-17 | 3.5 | 3.5 | 1 | 2.67 |
| OKG16-2 | 4 | 3 | 2 | 3.00 |
| OKG16-3 | 4 | 2 | 2.5 | 2.83 |
| OKG16-4 | 2 | 2.5 | 3 | 2.50 |
| OKG16-5 | 0.5 | 1.5 | 2.5 | 1.50 |
| OKG16-6 | 1.5 | 0 | 2 | 1.17 |
| OKG16-7 | 4 | 3 | 2.5 | 3.17 |
| OKG16-8 | 5 | 4.5 | 3.5 | 4.33 |
| OKG16-9 | 2 | 2 | 2 | 2.00 |
| SC 3-1 | 2.5 | 3.5 | 3.5 | 3.17 |
| SC 3-2 | 3.5 | 4 | 2.5 | 3.33 |
| SC3-3 | 2 | 3 | 1.5 | 2.17 |
| SC3-4 | 4 | 2 | 3.5 | 3.17 |
| SDRF16-1 | 0 | 1 | 0 | 0.33 |
| SDRF16-2 | 2.5 | 3 | 3 | 2.83 |
| SDRF16-4 | 2 | 2 | 3 | 2.33 |
| SDRF16-5 | 1.5 | 1.5 | 3.5 | 2.17 |
| SDRF16-6 | 2 | 2 | 2.5 | 2.17 |
| SDRF16-7 | 0 | 0.5 | 1 | 0.50 |
| SDRF16-8 | 0 | 0 | 0.00 |
| SDRF16-9 | 0.5 | 1 | 0 | 0.50 |
| Sn2000 | 4.5 | 3 | 4.5 | 4.00 |
| SN330NY91 | 1.5 | 2 | 2 | 1.83 |
| SN335NY91 | 2 | 1.5 | 0 | 1.17 |
| Reference | Value 1 | Value 2 | Value 3 | Value 4 |
|-----------|---------|---------|---------|---------|
| SN345NY91 | 1       | 0       | 0       | 0.33    |
| SN349NY91 | .       | 4       | 1.5     | 2.75    |
| SN351NY91 | 2.5     | 2.5     | 3       | 2.67    |
| SN356NY91 | 0       | 1       | 1       | 0.67    |
| SN358NY91 | 3       | 1.5     | 2.5     | 2.33    |
| SN366NY91 | 0       | 3       | 1.5     | 1.50    |
| SN369NY91 | 0       | 0       | 0       | 0.00    |
| SN377NY91 | 4       | 0.5     | 2       | 2.17    |
| Sn50      | 2       | 3       | 4.5     | 3.17    |
| Sn-6 (Sn69-1) | 0.5 | 0 | 3.5 | 1.33 |
| SnOre11-1 | 3.5 | 2.5 | 3 | 3.00 |
| SnOre11-2 | 3.5 | 3 | 3.5 | 3.33 |
| SnOre11-3 | 2.5 | 3.5 | 3.5 | 3.17 |
| SnOre11-4 | . | 4 | 3 | 3.50 |
| SnOre11-5 | 3.5 | 3.5 | 3 | 3.33 |
| SnOre11-6 | 3 | 3.5 | 4.5 | 3.67 |
| SnOre11-7 | 4.5 | 2.5 | 3.5 | 3.50 |
| SnOre11-8 | 4 | 2 | 2.5 | 2.83 |
| SNOV92X D1.3 | 2.5 | 2.5 | 3 | 2.67 |
| SNOV92X D4.1 | 3.5 | 3.5 | 2 | 3.00 |
| SNOV92X F1.1 | 4 | 2 | 2 | 2.67 |
| SNOV92X F2.1 | 3 | 3 | 2.5 | 2.83 |
| SNOV92X H1.4 | 3 | 3 | 3.5 | 3.17 |
| TN 5-1 | 4.5 | 5 | 3.5 | 4.33 |
| TN5-2 | 1.5 | 0 | 0.5 | 0.67 |
| TN5-3 | . | 0 | 1 | 0.50 |
| TN5-4 | 3.5 | 2.5 | 2.5 | 2.83 |
| TN5-5 | 4 | 3 | 3 | 3.33 |
| VA 5-2 | 2 | 2.5 | 3 | 2.50 |
| VA 5-3 | 3 | 3.5 | 4 | 3.50 |
| VA 5-4 | 3.5 | 3 | 3 | 3.17 |
| VA 5-5 | 4 | 0.5 | 3.5 | 2.67 |

*The “.” represents missing data.*
Table S3 Temporal expression of SnTox5 by Sn2000 in plant, on Lebsock.

| Time point (hours (h) post inoculation) | Expression of SnTox5 relative to actin | Standard error of the mean (SEM) |
|-----------------------------------------|----------------------------------------|---------------------------------|
| 4h                                      | 3.237055531                            | 0.32402                         |
| 12h                                     | 1.448097894                            | 0.08704                         |
| 24h                                     | 6.482131654                            | 1.29407                         |
| 48h                                     | 3.499210717                            | 0.26921                         |
| 72h                                     | 1.864790001                            | 0.26724                         |
| 96h                                     | 0.916129234                            | 0.05471                         |
| 120h                                    | 0.468878571                            | 0.03966                         |

Methods S1 Disease phenotyping

A set of 197 P. nodorum isolates was collected from geographically diverse winter, spring, and durum wheat growing regions of the US. The population consisted of 51 isolates collected from spring wheat in North Dakota and Minnesota, 45 isolates collected from durum wheat in North Dakota, nine isolates collected from winter wheat in South Dakota, and 92 isolates from winter wheat regions of the United States representing Arkansas, Georgia, Maryland, New York, North Carolina, Ohio, Oklahoma, Oregon, South Carolina, Tennessee, Texas, and Virginia, (Richards et al., 2019). Culture preparation and phenotyping was done as described by Friesen & Faris, (2012). In brief, a dried agar plug of each isolate was place on V8-PDA (150 ml of V8 juice, 10 g of Difco potato dextrose agar, 3g of CaCO3, 10g of Agar in 1000 ml of water) and allowed to rehydrate for 15 minutes. The rehydrated plug was then streaked across the plate to evenly distribute the spores and the plate was incubated at room temperature under continuous light for seven days or until pycnidia emerged. Plates with pycnidia were flooded with sterile-distilled water and agitated with a sterile inoculation loop to stimulate the release of pycnidiospores. Spores were harvested, and the spore concentration was adjusted to 1 × 10^6 spores/mL and two drops of Tween20 were added per 100 ml of spore suspension.

All isolates were phenotyped for disease reaction on LP29, the differential line for Snn5, which is the sensitivity gene targeted by SnTox5 (Friesen et al., 2012). LP29 is a progeny line chosen from the doubled haploid population derived from the cross Lebsock × PI94749 that segregated for the wheat susceptibility genes Snn5 and Tsn1 (Friesen et al., 2012). Each replicate consisted
of a single cone with three plants of LP29. Borders were planted with the wheat cultivar Alsen to reduce any edge effect. Plants were grown for approximately fourteen days. Plants at the two-to-three leaf stage were inoculated with a spore suspension using a pressurized paint sprayer. Leaves were inoculated until runoff and kept in a lighted mist chamber at 100% relative humidity at ~21 °C for 24 hours prior to being moved into a climate-controlled growth chamber at 21 °C with a 12-hour photoperiod for six additional days. At 7 days post-inoculation, disease was evaluated using a 0-5 rating scale based on the lesion type as described in Liu et al. (2004). Each experiment was performed in three replications and the average of the three replicates was used in downstream analysis.

Methods S2 Whole genome sequencing and variant identification
Raw sequencing reads for each isolate were generated using the Illumina HiSeq 4000 platform at BGI Americas Corp and uploaded to the NCBI short read archive under BioProject PRJNA398070 (Richards et al., 2019). Raw sequencing reads were trimmed using Trimmomatic v0.36 (Bolger et al., 2014) and were mapped to the reference genome sequence of *P. nodorum* isolate Sn2000 using BWA-MEM (Li, 2013). SAMtools `mpileup` (Li et al., 2009) was used to identify SNPs/InDels and the variants were filtered based on the genotype quality where only the polymorphisms with genotype quality equal to or greater than 40 with the support of a minimum of three reads were used for downstream analysis. All heterozygote calls were marked as missing data and variants with 30% or more missing data were removed from the dataset. In addition, markers with a minor allele frequency of less than 5% were filtered out from the final dataset used for genome-wide association study analysis.

Methods S3 Genome-wide association mapping
Mapping for GWAS was performed using GAPIT (Lipka et al., 2012; Tang et al., 2016) and TASSEL v5 (Bradbury et al., 2007). For the association mapping conducted using TASSEL v5, a naïve model and a model comprised of the first three components of PCA as fixed effects were used. For the analysis performed with GAPIT, models with a kinship matrix (K) using EMMA as a random effect and models using a combination of both PCA and K were used. The most robust model was selected based on Q-Q plot results. A Bonferroni correction was used to adjust the *P-*
value in the R statistical environment and the markers were considered significant when an adjusted $P$-value was equal to or less than 0.05.

**Methods S4 QTL analysis of the LP749 population using SnTox5 gene disruption strains**

The LP749 population (Friesen et al., 2012) was used to map the SnTox5-Snn5 interaction. The same population was inoculated separately with the two SnTox5 gene disruption strains Sn2kΔTox5-10 and Sn2kΔTox5-15, the ectopic strain Sn2k-ect7, and the wild type strain Sn2000. Side by side inoculations using each of the four strains were completed on full LP749 populations and the disease was evaluated at seven days post-inoculation as described above. Averages disease reactions from three replicates were used to perform composite interval mapping (CIM) to evaluate the significance of the SnTox5-Snn5 and SnToxA-Tsn1 interactions for the inoculation of each *P. nodorum* strain using Qgene v4.4.0 (Joehanes & Nelson, 2008). A permutation test that consisted of 1000 iterations yielded a LOD threshold of 3.0 at an experimental-wise significance level of 0.05 and was used to evaluate the significance of the resulting QTL.

**Methods S5 Development of construct of SnTox5 using Gateway cloning system**

The Gateway cloning system (Gong et al., 2015) was used to develop constructs with SnTox5. Approximately 1.7 kb of the genomic region of SnTox5, including a 1 kb region upstream of the gene that included the putative promotor region (Fig. S1) was amplified with forward primer SnTox5_DONOR_F and reverse primer SnTox5_DONOR_R (Table S1). Each primer consisted of a full length attB sequence at the 5’ end. The PCR amplicon with an attB sequence at the end was visualized using gel electrophoresis and purified using the GeneJet Gel Extraction Kit (Thermo Scientific). Fragments were cloned into the pDONOR vector via a BP Clonase reaction (Invitrogen). The pDONOR vector, containing the resistance gene zeocin, was transformed into *E. coli* and transformed colonies were selected on low salt Luria-Bertani broth (LB) agar medium (10g of tryptone, 5g of NaCl, 5g of yeast extract, and 16g of agar in 1000 ml of water) amended with zeocin (50 µg/ml). Five *E. coli* transformants were picked and inoculated in 2 ml of low salt LB with zeocin and used to extract plasmid using the Monarch plasmid miniprep kit (New England Bio Labs). Presence of the genomic fragment containing SnTox5 was verified by Sanger sequencing using the Tox5_Seq_F, M13 forward and reverse primers (Supplementary
Table 1). The extracted pDONOR plasmid with the insertion was used to perform an LR Clonase reaction as instructed by the manufacturer (Invitrogen) to transfer the genomic region into the destination vector, pFPL-RH, that contained the hygromycin resistance cassette.

**Methods S6 Inoculation and infiltration of gain-of-function transformants and culture filtrates on to LP749 population**

Sn79+Tox5-3 and Sn79-1087 were inoculated onto the LP749 population side by side and QTL analysis was done as mentioned previously. Furthermore, culture filtrates of Sn79+Tox5-3 and Sn79-1087 were prepared as described (Liu et al., 2004) and used to infiltrate the LP749 population. Sensitivity was scored using a 0-3 rating scale where 0 was insensitive and 3 was highly sensitive (Friesen & Faris 2012). Data was used for QTL analysis as described in Method S3.

**Methods S7 Extraction of SnTox5 sequence for population genetics and haplotype analysis**

BAM files for 197 isolates of the GWAS panel were developed as described above and were used to extract reads mapped to chromosome 8:53219-53872bp of the Sn2000 genome using SAMtools (Li et al., 2009) and a BED file, creating FastQ files for each isolate. De-novo assembly of SnTox5 for each isolate was completed using SPADES v.3.11.1 (Nurk et al., 2013) with default settings and SnTox5 sequences for each isolate were developed for use in haplotype analysis. In addition, coverage of the SnTox5 gene in each isolate was calculated using the ‘coverage’ function of BEDTools (Quinlan et al., 2010). Isolates with more than 50% of the SnTox5 gene were considered to have the gene, whereas, isolates with coverage less than or equal to 50% were considered to lack the gene. Genomic sequences of SnTox5 for isolates that contained complete coverage of the gene were converted to FASTA format and imported into DNASP v6 for population genetic analysis.

**Methods S8 Development of Snn5 mutants of LP29**

The Snn5 differential line, LP29, which carries the Snn5 allele from Lebsock, was used for mutagenesis to generate LP29ems lines. LP29 seeds were treated with 0.25% ethyl methanesulfonate (EMS) in 0.05 M phosphate buffer as described in Williams et al., (1992). 585 M2 families were infiltrated with Sn2000K06-1 (Friesen et al., 2006) culture filtrates containing
SnTox5. Ten to fourteen $M_2$ individuals per $M_1$ were evaluated. Plants were scored for presence or absence of necrosis five days after infiltration. SnTox5-insensitive mutants from five $M_2$ families were self-pollinated to obtain $M_3$. LP29 $M_3$ families were infiltrated with Sn79+Tox5-3 culture filtrates to confirm insensitivity. $M_3$ and $M_4$ plants from the LP29 mutant line LP29emsp931, hereafter designated LP29Δsnn5 were used in this study. The Snn5 gene was cloned and validated via mutagenesis, and the details will be published elsewhere (K.L.D. Running and J.D. Faris, personal communication).

References

Bolger, A.M., Lohse, M. and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30: 2114-2120.

Bradbury, P.J., Zhang, Z., Kroon, D.E., Casstevens, T.M., Ramdoss, Y. and Buckler, E.S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics 23:2633-2635.

Friesen, T.L., Stukenbrock, E.H., Liu, Z., Meinhardt, S., Ling, H., Faris, J.D., Rasmussen, J.B., Solomon, P.S., McDonald, B.A., and Oliver, R.P. (2006). Emergence of a new disease as a result of interspecific virulence gene transfer. Nat. Genet. 38:953-956.

Friesen, T.L., Chu, C., Xu, S.S., and Faris, J.D. (2012). SnTox5-Snn5: a novel Stagonospora nodorum effector-wheat gene interaction and its relationship with the SnToxA-Tsn1 and SnTox3-Snn3-B1 interactions. Mol. Plant Pathol. 13:1101-1109.

Friesen, T.L., and Faris, J.D. (2012). Characterization of plant-fungal interactions involving necrotrophic effector-producing plant pathogens. In Plant fungal pathogens, M.D. Bolton, and B.P.H.J. Thomma, eds ( Totowa, NJ, United State: Humana Press), pp.191-207.

Gong, X., Hurtado, O., Wang, B., Wu, C., Yi, M., Giraldo, M., Valent, B., Goodin, M., and Farman, M. (2015). pFPL vectors for high-throughput protein localization in fungi: detecting cytoplasmic accumulation of putative effector proteins. Mol. Plant-Microbe Interact. 28:107-121.

Joehanes, R., and Nelson, J.C. (2008). QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24:2788-2789.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G. and Durbin, R. (2009). The sequence alignment/map format and SAMtools. Bioinformatics 25:2078-2079.
Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv preprint arXiv:1303.3997.

Lipka, A.E., Tian, F., Wang, Q., Peiffer, J., Li, M., Bradbury, P.J., Gore, M.A., Buckler, E.S., and Zhang, Z. (2012). GAPIT: genome association and prediction integrated tool. Bioinformatics 28:2397-2399.

Liu, Z.H., Faris, J.D., Meinhardt, S.W., Ali, S., Rasmussen, J.B., and Friesen, T.L. (2004). Genetic and physical mapping of a gene conditioning sensitivity in wheat to a partially purified host-selective toxin produced by Stagonospora nodorum. Phytopathology 94:1056-1060.

Nurk, S., Bankevich, A., Antipov, D., Gurevich, A., Korobeynikov, A., Lapidus, A., Prjibelsky, A., Pyshkin, A., Sirotkin, A., Sirotkin, Y. and Stepanauskas, R., (2013). Assembling genomes and mini-metagenomes from highly chimeric reads. In Annual International Conference on Research in Computational Molecular Biology, M. Deng, R. Jiang, F. Sun, and Y.-Y. Zhang, eds (Berlin, Heidelberg, Germany: Springer), pp. 158-170.

Quinlan, A.R., and Hall, I.M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26: 841-842.

Richards, J.K., Stukenbrock, E.H., Carpenter, J., Liu, Z., Cowger, C., Faris, J.D. and Friesen, T.L. (2019). Local adaptation drives the diversification of effectors in the fungal wheat pathogen Parastagonospora nodorum in the United States. PLoS Genet. 15: e1008223.

Tang, Y., Liu, X., Wang, J., Li, M., Wang, Q., Tian, F., Su, Z., Pan, Y., Liu, D., Lipka, A.E. and Buckler, E.S., (2016). GAPIT version 2: an enhanced integrated tool for genomic association and prediction. Plant Genome 9:1-9.

Williams, N.D., Miller, J.D., and Klindworth, D.L. (1992). Induced mutations of a genetic suppressor of resistance to wheat stem rust. Crop Sci. 32:612-616.