

### Supplemental Tables and Figures

**Table S1** Summary of the sequencing data generated for transcriptomes of wild type and TaNACL-D1-overexpressing cv. Fielder for given conditions after read filtering and genome mapping.

| Condition/sample <sup>1</sup> | Clean reads | Total mapped (%) <sup>2</sup> | Uniquely mapped (%) <sup>3</sup> | Q20(%) |
|-------------------------------|-------------|-------------------------------|----------------------------------|--------|
| WT_Fg_rep1_1dpi               | 49443988    | 83.9                          | 55.6                             | 96     |
| WT_Fg_rep2_1dpi               | 49526014    | 86.2                          | 56.2                             | 96     |
| WT_Fg_rep3_1dpi               | 49585466    | 86.5                          | 56.9                             | 96     |
| OE_Fg_rep1_1dpi               | 48477590    | 87                            | 59.8                             | 96     |
| OE_Fg_rep2_1dpi               | 49496904    | 86.6                          | 57.2                             | 96     |
| OE_Fg_rep3_1dpi               | 49742842    | 86.6                          | 57.2                             | 96     |
| WT_Tween20_rep1_1dpi          | 49969834    | 85.1                          | 52.7                             | 96     |
| WT_Tween20_rep2_1dpi          | 48481434    | 85.5                          | 53.5                             | 97     |
| WT_Tween20_rep3_1dpi          | 48822502    | 85.4                          | 53.5                             | 97     |
| OE_Tween20_rep1_1dpi          | 48335298    | 85.3                          | 53.3                             | 97     |
| OE_Tween20_rep2_1dpi          | 50087594    | 85.2                          | 53.3                             | 97     |
| OE_Tween20_rep3_1dpi          | 50185508    | 85.5                          | 53.3                             | 97     |
| WT_rep1_0dpi_NT               | 48444492    | 85.4                          | 53.1                             | 97     |
| WT_rep2_0dpi_NT               | 48232922    | 85.5                          | 52.7                             | 97     |
| WT_rep3_0dpi_NT               | 48449112    | 85.4                          | 52.3                             | 97     |
| OE_rep1_0dpi_NT               | 50045512    | 85.6                          | 52.7                             | 96     |
| OE_rep2_0dpi_NT               | 48691474    | 85.9                          | 53.5                             | 97     |
| OE_rep3_0dpi_NT               | 48585640    | 85.4                          | 52.8                             | 97     |

<sup>1</sup> WT= Wild type; OE = *TaNACL-D1* overexpressor; Fg = *Fusarium graminearum* treatment; Tween20 = mock treatment; NT = no treatment; dpi = days post-inoculation; Rep1-3 = replicates 1-3. <sup>2</sup>Percentage of fragments that could be mapped to target sequences out of the total number of processed paired-end clean reads. <sup>3</sup>Percentage of fragments that could be mapped to a unique target sequence out of the total number of processed paired-end clean reads.

**Table S2** The total number of TaNACL-D1-regulated genes and the total number of corresponding TaNACL-D1-regulated transcripts in non-treated plants (day zero), Tween20 (mock)-treated plants at one day post-inoculation (dpi), and *Fusarium graminearum*-treated plants at one dpi; and the total number of *F. graminearum*-regulated transcripts and genes in the TaNACL-D1-overexpressing cv. Fielder or wild type.

| Comparison <sup>1</sup> | Nr of DEGs <sup>2</sup> | Nr of DETs <sup>3</sup> |
|-------------------------|-------------------------|-------------------------|
| NT_0dpi_OEvsWT          | 264                     | 272                     |
| Tween20_1dpi_OEvsWT     | 167                     | 172                     |
| Fg_1dpi_OEvsWT          | 140                     | 147                     |
| WT_1dpi_FgvsTween20     | 6452                    | 6704                    |
| OE_1dpi_FgvsTween20     | 8322                    | 8704                    |

<sup>1</sup> WT= Wild type; OE = *TaNACL-D1* overexpressor; Fg = *Fusarium graminearum* treatment; Tween20 = mock treatment; NT = no treatment; dpi = days post inoculation; vs = versus. <sup>2</sup> Nr= number; DEGs= differentially expressed genes. <sup>3</sup> DETs = differentially expressed transcripts.

**Table S3** Total number of gene ontology terms per three main gene ontology categories that were enriched in associated *Fusarium graminearum*-up/downregulated transcripts at one day post-inoculation in TaNACL-D1-overexpressing cv. Fielder and wild type.

| GO category          | Number of upregulated GO-terms <sup>1</sup> |                 | Number of downregulated GO-terms <sup>1</sup> |                 |
|----------------------|---|-----------------|---|-----------------|
|                      | OE <sup>1</sup>                             | WT <sup>1</sup> | OE <sup>1</sup>                               | WT <sup>1</sup> |
| Biological process   | 358   | 315             | 169   | 36              |
| Molecular function   | 232   | 218             | 77  | 42              |
| Cellular compartment | 23  | 20              | 32  | 4               |

<sup>1</sup> GO= Gene ontology; OE = *TaNACL-D1*-overexpressing cv. Fielder; WT = wild type.

**Table S4** Biological processes enriched in *Fusarium graminearum*-up/downregulated transcripts in the TaNACL-D1-overexpressing cv. Fielder and wild type at one day post-inoculation.

| Biological process <sup>2</sup>                                   | OE <sup>1</sup>  |  |   |                               |             | WT <sup>1</sup>  |  |   |                               |  |
|---|------------------|--|---|-------------------------------|-------------|------------------|--|---|-------------------------------|--|
|   | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> |             | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> |  |
| <b>ABSCISIC ACID</b>  |                  |  |   |                               |             |                  |  |   |                               |  |
| Response to abscisic acid   | -                | -  | -   | -                             | 0.036748995 | 70               | 596  | 0.1   |                               |  |
| <u>Positive regulation of abscisic acid biosynthetic process</u>  | 0.002189114      | 3  | 4   | 0.8                           | -           | -                | -  | -   | -                             |  |
| <b>SALICYLIC ACID</b>   |                  |  |   |                               |             |                  |  |   |                               |  |
| Cellular response to salicylic acid stimulus                      | 3.1E-05          | 22   | 69  | 0.3                           | 0.000253    | 18               | 69   | 0.3   |                               |  |
| Positive regulation of salicylic acid mediated signalling pathway | 0.002543         | 4  | 4   | 1                             | 0.035366    | 3                | 4  | 0.8   |                               |  |
| Regulation of salicylic acid biosynthetic process                 | 2.18E-06         | 17   | 38  | 0.4                           | 4.62E-05    | 13               | 34   | 0.4   |                               |  |
| Regulation of salicylic acid mediated signalling pathway          | 3.88E-05         | 18   | 50  | 0.4                           | 0.001131    | 14               | 51   | 0.3   |                               |  |
| Regulation of salicylic acid metabolic process                    | 2.67E-07         | 21   | 50  | 0.4                           | 7.56E-05    | 15               | 46   | 0.3   |                               |  |
| Response to salicylic acid  | 2.59E-12         | 48   | 142   | 0.3                           | 2.39E-09    | 37               | 135  | 0.3   |                               |  |
| <u>Salicylic acid biosynthetic process</u>                        | 5.19E-06         | 17   | 40  | 0.4                           | 9.46E-05    | 13               | 36   | 0.4   |                               |  |

Table S4 continued...

| OE <sup>1</sup>   |                  |  |   |                               | WT <sup>1</sup>  |  |   |                               |  |
|---|------------------|--|---|-------------------------------|------------------|--|---|-------------------------------|--|
| Biological process <sup>2</sup>                                     | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> |  |
| Salicylic acid mediated signalling pathway                          | 1.74E-05         | 20   | 57  | 0.4                           | 0.00035          | 16   | 58  | 0.3                           |  |
| Salicylic acid metabolic process                                    | 1.16E-15         | 41   | 90  | 0.5                           | 8.53E-13         | 33   | 86  | 0.4                           |  |
| JASMONIC ACID   |                  |  |   |                               |                  |  |   |                               |  |
| Response to jasmonic acid   | 1.28E-19         | 67   | 180   | 0.4                           | 3.49E-11         | 46   | 172   | 0.3                           |  |
| Jasmonic acid mediated signalling pathway                           | 6.06E-10         | 40   | 121   | 0.3                           | 0.000259         | 25   | 117   | 0.2                           |  |
| Cellular response to jasmonic acid stimulus                         | 5.28E-09         | 40   | 129   | 0.3                           | 0.000658         | 25   | 124   | 0.2                           |  |
| Regulation of jasmonic acid mediated signalling pathway             | 2.25E-13         | 33   | 69  | 0.5                           | 5.26E-08         | 23   | 66  | 0.3                           |  |
| Jasmonic acid metabolic process                                     | 7.06E-09         | 29   | 76  | 0.4                           | 3.57E-06         | 22   | 75  | 0.3                           |  |
| Jasmonic acid biosynthetic process                                  | 0.024225         | 9  | 28  | 0.3                           | -                | -  | -   | -                             |  |
| GIBBERELLI C ACID   |                  |  |   |                               |                  |  |   |                               |  |
| Gibberellic acid mediated signalling pathway                        | 9.9E-06          | 24   | 75  | 0.3                           | 0.00023          | 19   | 75  | 0.3                           |  |
| Negative regulation of gibberellic acid mediated signalling pathway | 1.54E-16         | 22   | 26  | 0.8                           | 4.59E-14         | 18   | 23  | 0.8                           |  |
| Regulation of gibberellic acid mediated signalling pathway          | 1.01E-08         | 22   | 47  | 0.5                           | 3.51E-07         | 18   | 46  | 0.4                           |  |
| ETHYLENE  |                  |  |   |                               |                  |  |   |                               |  |
| Response to ethylene  | 7.67E-19         | 80   | 249   | 0.3                           | 1.89E-12         | 58   | 235   | 0.2                           |  |
| Ethylene-activated signalling pathway                               | 1.43E-12         | 54   | 170   | 0.3                           | 9.89E-08         | 38   | 160   | 0.2                           |  |

Table S4 continued...

| Biological process <sup>2</sup>  | OE <sup>1</sup>  |  |   |                               | WT <sup>1</sup>  |  |   |                               |
|--|------------------|--|---|-------------------------------|------------------|--|---|-------------------------------|
|  | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> |
| Cellular response to ethylene stimulus<br><b>INDOLE-ACETIC ACID</b>                    | 5.18E-12         | 54   | 175   | 0.3                           | 1.99E-07         | 38   | 164   | 0.2                           |
| Indole-containing compound metabolic process   | 1.57E-22         | 60   | 133   | 0.5                           | 4.84E-21         | 52   | 130   | 0.4                           |
| Indole-containing compound biosynthetic process<br><b>PHENYLPROPANOIC ACID PATHWAY</b> | 8.16E-25         | 56   | 108   | 0.5                           | 6.56E-23         | 49   | 107   | 0.5                           |
| Phenylpropanoid metabolic process  | 1.56E-41         | 107  | 231   | 0.5                           | 1.18E-40         | 96   | 230   | 0.4                           |
| Phenylpropanoid biosynthetic process<br><b>DETOXIFICATION</b>                          | 1.13E-34         | 79   | 154   | 0.5                           | 1.54E-32         | 70   | 154   | 0.5                           |
| Cellular detoxification  | 3.82E-13         | 119  | 548   | 0.2                           | 1.96E-14         | 106  | 550   | 0.2                           |
| Cellular oxidant detoxification  | 1.95E-11         | 109  | 513   | 0.2                           | 2E-13            | 99   | 515   | 0.2                           |
| Cellular detoxification of aldehyde  | 0.014544         | 10   | 31  | 0.3                           | -                | -  | -   | -                             |

<sup>1</sup> OE = *Ta*NAC1-D1-overexpressing cv. Fielder; WT = wild type.

<sup>2</sup> Underlined biological process is downregulated.

<sup>3</sup> FDR = False discovery rate (Fischer's exact test, FDR < 0.05).

<sup>4</sup> Reference = The number of all expressed transcripts associated with the biological process in the corresponding genotype.

<sup>5</sup> Transcript ratio = The number of *F. graminearum*-regulated transcripts associated with the biological process relative to the number of transcripts associated with the same biological process in the reference (the number of all expressed transcripts associated with the biological process in the corresponding genotype).

**Table S5** *Fusarium graminearum*-upregulated biological processes that are common to TaNACL-D1-overexpressing cv. Fielder and wild type at one day post-inoculation.

| Biological processes                            | Number of transcripts in the reference (OE) <sup>1,2</sup> | Transcript ratio (OE) <sup>1,3</sup> | Number of transcripts in the reference (WT) <sup>1,2</sup> | Transcript ratio (WT) <sup>1,3</sup> | Ratio of Transcript ratios in OE compared to WT <sup>1,4</sup> |
|---|--|--------------------------------------|--|--------------------------------------|--|
| Carboxylic acid metabolic process               | 2662   | 0.2                                  | 2656   | 0.1                                  | 1.2  |
| Cell communication                              | 3015   | 0.2                                  | 3008   | 0.1                                  | 1.2  |
| Cellular protein modification process           | 8586   | 0.1                                  | 8582   | 0.1                                  | 1.3  |
| Cellular response to chemical stimulus          | 2013   | 0.2                                  | 2004   | 0.1                                  | 1.2  |
| Cellular response to stimulus                   | 4868   | 0.1                                  | 4864   | 0.1                                  | 1.2  |
| Defence response                                | 2020   | 0.2                                  | 2000   | 0.2                                  | 1.2  |
| Defence response to other organism              | 962  | 0.3                                  | 955  | 0.2                                  | 1.2  |
| Interspecies interaction                        | 962  | 0.3                                  | 1120   | 0.2                                  | 1.3  |
| Immune response                                 | 962  | 0.3                                  | 955  | 0.2                                  | 1.2  |
| Immune system process                           | 1132   | 0.3                                  | 955  | 0.2                                  | 1.1  |
| Macromolecule modification                      | 9305   | 0.1                                  | 9304   | 0.1                                  | 1.3  |
| Organic acid biosynthetic process               | 1316   | 0.2                                  | 1323   | 0.2                                  | 1.2  |
| Organic acid metabolic process                  | 2771   | 0.2                                  | 2766   | 0.1                                  | 1.2  |
| Organonitrogen compound metabolic process       | 16126  | 0.1                                  | 16128  | 0.1                                  | 1.2  |
| Oxoacid metabolic process                       | 2694   | 0.2                                  | 2687   | 0.1                                  | 1.2  |
| Phosphate-containing compound metabolic process | 7455   | 0.1                                  | 7475   | 0.1                                  | 1.2  |
| Phosphorus metabolic process                    | 7551   | 0.1                                  | 7575   | 0.1                                  | 1.2  |
| Phosphorylation                                 | 5198   | 0.2                                  | 5227   | 0.1                                  | 1.2  |
| Protein modification process                    | 8586   | 0.1                                  | 8582   | 0.1                                  | 1.3  |
| Protein phosphorylation                         | 3969   | 0.2                                  | 3972   | 0.2                                  | 1.2  |
| Response to biotic stimulus                     | 1160   | 0.3                                  | 1151   | 0.3                                  | 1.2  |
| Response to chemical                            | 3306   | 0.2                                  | 3284   | 0.1                                  | 1.2  |
| Response to endogenous stimulus                 | 1752   | 0.2                                  | 1724   | 0.1                                  | 1.3  |
| Response to external biotic stimulus            | 1065   | 0.3                                  | 1055   | 0.2                                  | 1.2  |
| Response to external stimulus                   | 1425   | 0.2                                  | 1415   | 0.2                                  | 1.2  |
| Response to hormone                             | 1733   | 0.2                                  | 1705   | 0.1                                  | 1.3  |
| Response to organic substance                   | 2210   | 0.2                                  | 2185   | 0.1                                  | 1.3  |
| Response to other organism                      | 1065   | 0.3                                  | 1055   | 0.2                                  | 1.2  |
| Response to stimulus                            | 8495   | 0.1                                  | 8476   | 0.1                                  | 1.2  |
| Response to stress                              | 4996   | 0.1                                  | 4968   | 0.1                                  | 1.2  |
| Signal transduction                             | 2675   | 0.2                                  | 2677   | 0.1                                  | 1.2  |
| Signalling                                      | 2680   | 0.2                                  | 2682   | 0.1                                  | 1.2  |
| Small molecule biosynthetic process             | 1826   | 0.2                                  | 1836   | 0.1                                  | 1.2  |
| Small molecule metabolic process                | 4484   | 0.1                                  | 4484   | 0.1                                  | 1.2  |



**Table S5** continued...

| Biological processes             | Number of transcripts in the reference (OE) <sup>1,2</sup> | Transcript ratio (OE) <sup>1,3</sup> | Number of transcripts in the reference (WT) <sup>1,2</sup> | Transcript ratio (WT) <sup>1,3</sup> | Ratio of Transcript ratios in OE compared to WT <sup>1,4</sup> |
|----------------------------------|--|--------------------------------------|--|--------------------------------------|--|
| Small molecule metabolic process | 4484   | 0.1                                  | 4484   | 0.1                                  | 1.2  |
| Transmembrane transport          | 3458   | 0.1                                  | 3446   | 0.1                                  | 1.2  |

<sup>1</sup>OE = *TaNAACL-D1*-overexpressing cv. Fielder; WT = wild type.

<sup>2</sup>Reference = All expressed transcripts in the corresponding genotype.

<sup>3</sup>Transcript ratio = The number of *F. graminearum*-regulated transcripts associated with the biological process relative to the number of transcripts associated with the same biological process in the reference (all expressed transcripts in the corresponding genotype).

<sup>4</sup>Ratio of the transcript ratios = The transcript ratio values compared between genotypes by dividing the value from the OE with the value from the WT.

**Table S6** *Fusarium graminearum*-downregulated biological processes that are common to TaNACL-D1-overexpressing cv. Fielder and wild type at one day post-inoculation.

| Biological processes                             | Number of transcripts in the reference (OE) <sup>1,2</sup> | Transcript ratio (OE) <sup>1,3</sup> | Number of transcripts in the reference (WT) <sup>1,2</sup> | Transcript ratio (WT) <sup>1,3</sup> | Ratio of Transcript ratios in OE compared to WT <sup>1,4</sup> |
|--|--|--------------------------------------|--|--------------------------------------|--|
| (1->3)-beta-D-glucan biosynthetic process        | 44   | 0.16                                 | 45   | 0.11                                 | 1.43   |
| Actin cytoskeleton organization                  | 288  | 0.10                                 | 281  | 0.05                                 | 2.25   |
| Actin filament organization                      | 271  | 0.10                                 | 264  | 0.05                                 | 2.10   |
| Actin filament-based movement                    | 48   | 0.17                                 | 45   | 0.13                                 | 1.25   |
| Actin filament-based process                     | 291  | 0.10                                 | 284  | 0.05                                 | 2.25   |
| Actin filament-based transport                   | 48   | 0.17                                 | 45   | 0.13                                 | 1.25   |
| Anatomical structure morphogenesis               | 740  | 0.06                                 | 750  | 0.03                                 | 1.75   |
| Cell morphogenesis                               | 305  | 0.08                                 | 314  | 0.05                                 | 1.61   |
| Cell plate assembly                              | 20   | 0.30                                 | 20   | 0.25                                 | 1.20   |
| Chromosome organization                          | 1746   | 0.05                                 | 1768   | 0.02                                 | 1.84   |
| Cytoskeleton-dependent intracellular transport   | 61   | 0.13                                 | 57   | 0.11                                 | 1.25   |
| Demethylation                                    | 95   | 0.12                                 | 99   | 0.08                                 | 1.43   |
| DNA demethylation                                | 39   | 0.13                                 | 39   | 0.13                                 | 1.00   |
| Establishment of localization in cell            | 2208   | 0.04                                 | 2202   | 0.02                                 | 1.53   |
| Establishment of vesicle localization            | 106  | 0.09                                 | 105  | 0.08                                 | 1.24   |
| Intracellular transport                          | 2131   | 0.04                                 | 2123   | 0.02                                 | 1.50   |
| Movement of cell or subcellular component        | 252  | 0.09                                 | 244  | 0.05                                 | 2.02   |
| Nuclear transport                                | 386  | 0.07                                 | 380  | 0.05                                 | 1.48   |
| Nucleocytoplasmic transport                      | 386  | 0.07                                 | 380  | 0.05                                 | 1.48   |
| Protein import into nucleus                      | 172  | 0.08                                 | 169  | 0.05                                 | 1.42   |
| Regulation of anatomical structure morphogenesis | 146  | 0.12                                 | 148  | 0.06                                 | 2.03   |
| Regulation of autophagy                          | 29   | 0.21                                 | 27   | 0.15                                 | 1.40   |
| Regulation of cell morphogenesis                 | 106  | 0.15                                 | 109  | 0.08                                 | 1.83   |
| Regulation of cell shape                         | 89   | 0.18                                 | 90   | 0.10                                 | 1.80   |
| Vesicle cytoskeletal trafficking                 | 48   | 0.17                                 | 45   | 0.13                                 | 1.25   |
| Vesicle localization                             | 106  | 0.09                                 | 105  | 0.08                                 | 1.24   |
| Vesicle transport along actin filament           | 48   | 0.17                                 | 45   | 0.13                                 | 1.60   |

<sup>1</sup> OE = *TaNACL-D1*-overexpressing cv. Fielder; WT = wild type.

<sup>2</sup> Reference = All expressed transcripts in the corresponding genotype.

<sup>3</sup> Transcript ratio = The number of *F. graminearum*-regulated transcripts associated with the biological process relative to the number of transcripts associated with the same biological process in the reference (all expressed transcripts in the corresponding genotype).

<sup>4</sup> Ratio of the transcript ratios = The transcript ratio values compared between genotypes by dividing the value from the OE with the value from the WT.

**Table S7** *Fusarium graminearum*-induced *Triticum aestivum* transcripts with putative function in cell-wall enforcement in the *TaNAACL-D1*-overexpressing cv. Fielder as compared to the wild type.

| Transcript ID <sup>1</sup> | Description based on the top <i>Viridiplantae</i> BLAST hit | Wild type, <i>F. graminearum</i> | <i>F. graminearum</i> response | <i>TaNAACL-D1</i> -overexpressor, <i>F. graminearum</i> | <i>F. graminearum</i> response |
|----------------------------|---|----------------------------------|--------------------------------|---|--------------------------------|
|                            |   | Fold change <sup>2</sup>         | False discovery rate           | Fold change <sup>2</sup>                                | False discovery rate           |
| TraesCS2A02G490000.1       | Agmatine coumaroyltransferase                               | 26.2                             | 0.002016799                    | <b>136.6</b>  | 3.09351E-05                    |
| TraesCS2A02G490700.1       | Agmatine coumaroyltransferase                               | <b>402.1</b>                     | 3.20747E-21                    | 383.9   | 6.77792E-44                    |
| TraesCS2B02G518300.1       | Agmatine coumaroyltransferase                               | 13.3                             | 5.5981E-07                     | <b>15.7</b>   | 2.0043E-05                     |
| TraesCS2B02G518900.1       | Agmatine coumaroyltransferase                               | 33.8                             | 5.94079E-24                    | <b>48.6</b>   | 2.76263E-69                    |
| TraesCS2B02G519100.1       | Agmatine coumaroyltransferase                               | 17.8                             | 2.42961E-22                    | <b>26.4</b>   | 2.98783E-56                    |
| TraesCS2D02G490900.1       | Agmatine coumaroyltransferase                               | 91.3                             | 2.06536E-06                    | <b>104.1</b>  | 1.22003E-11                    |
| TraesCS2D02G491100.1       | Agmatine coumaroyltransferase                               | 80.0                             | 4.02995E-42                    | <b>118.8</b>  | 3.3154E-99                     |
| TraesCS1A02G088300.1       | Tryptamine hydroxycinnamoyltransferase 2-like               | 63.8                             | 7.47387E-08                    | <b>79.4</b>   | 1.75298E-19                    |
| TraesCS1B02G107300.1       | Tryptamine hydroxycinnamoyltransferase 2-like               | <b>194.9</b>                     | 1.79332E-05                    | 148.3   | 3.25294E-07                    |
| TraesCS1B02G107400.1       | Tryptamine hydroxycinnamoyltransferase 2-like               | <b>8.8</b>                       | 0.000328716                    | 8.7   | 7.4906E-08                     |
| TraesCS1B02G107500.1       | Tryptamine hydroxycinnamoyltransferase 2-like               | 13.0                             | 0.000122439                    | <b>14.4</b>   | 1.13826E-09                    |
| TraesCS1D02G089900.1       | Tryptamine hydroxycinnamoyltransferase 2-like               | 3.8                              | 0.007365152                    | <b>4.6</b>  | 1.52848E-10                    |
| TraesCS2A02G354500.1       | Hydroxycinnamoyltransferase 1                               | 2.1                              | 7.9669E-10                     | <b>2.4</b>  | 1.17209E-09                    |
| TraesCS2D02G354300.1       | Hydroxycinnamoyltransferase 1                               | -                                | -                              | 2.6   | 8.20196E-12                    |
| TraesCS5A02G337500.1       | Putrescine hydroxycinnamoyltransferase 3-like               | 210.3                            | 1.32217E-30                    | <b>644.1</b>  | 8.01383E-39                    |
| TraesCS5A02G337700.1       | Putrescine hydroxycinnamoyltransferase 3-like               | 149.0                            | 4.12867E-06                    | <b>199.8</b>  | 7.7686E-08                     |
| TraesCS5A02G337800.1       | Putrescine hydroxycinnamoyltransferase 3-like               | 83.7                             | 2.89268E-07                    | <b>362.5</b>  | 3.2175E-10                     |
| TraesCS5A02G337900.1       | Putrescine hydroxycinnamoyltransferase 3-like               | 38.7                             | 0.000330941                    | <b>220.7</b>  | 4.19648E-08                    |
| TraesCS5A02G338600.1       | putrescine hydroxycinnamoyltransferase-like                 | 14.0                             | 4.21843E-10                    | <b>25.2</b>   | 6.19772E-12                    |
| TraesCS5B02G304300.1       | Putrescine hydroxycinnamoyltransferase 3-like               | <b>69.2</b>                      | 1.1235E-10                     | 55.2  | 2.06221E-17                    |
| TraesCS5B02G336300.1       | Putrescine hydroxycinnamoyltransferase 3-like               | 118.5                            | 1.83358E-20                    | 118.5   | 8.53327E-38                    |
| TraesCS5B02G336500.1       | Putrescine hydroxycinnamoyltransferase 3-like               | 119.7                            | 2.15903E-06                    | <b>334.7</b>  | 6.90041E-10                    |
| TraesCS5B02G336600.1       | Putrescine hydroxycinnamoyltransferase 3-like               | <b>312.9</b>                     | 1.57513E-08                    | 130.5   | 1.49004E-09                    |

Table S7 continued...

| Transcript ID <sup>1</sup> | Description based on the top <i>Viridiplantae</i> BLAST hit | Wild<br><i>graminearum</i>  | type,<br>response          | <i>F.</i><br><i>TaNAACL-D1-</i><br>overexpressor,<br><i>graminearum</i> | <i>F.</i><br>response      |
|----------------------------|---|-----------------------------|----------------------------|---|----------------------------|
|                            |   | Fold<br>change <sup>2</sup> | False<br>discovery<br>rate | Fold<br>change <sup>2</sup>   | False<br>discovery<br>rate |
| TraesCS5B02G336700.1       | Putrescine<br>hydroxycinnamoyltransferase<br>3-like         | 12.9                        | 1.2434E-07                 | <b>18.1</b>   | 3.87003E-11                |
| TraesCS5B02G337000.1       | Putrescine<br>hydroxycinnamoyltransferase-<br>like          | 19.2                        | 9.60135E-07                | <b>29.2</b>   | 1.12996E-08                |
| TraesCS5D02G310800.1       | Putrescine<br>hydroxycinnamoyltransferase<br>3-like         | -                           | -                          | 67.9  | 0.017339424                |
| TraesCS5D02G342100.1       | Putrescine<br>hydroxycinnamoyltransferase<br>3-like         | 130.1                       | 8.33446E-40                | <b>160.2</b>  | 9.99816E-85                |
| TraesCS5D02G342300.1       | Putrescine<br>hydroxycinnamoyltransferase<br>3-like         | <b>261.3</b>                | 5.24071E-07                | 68.0  | 5.00151E-09                |
| TraesCS5D02G342400.1       | Putrescine<br>hydroxycinnamoyltransferase<br>3-like         | `                           | \                          | <b>194.7</b>  | 3.37646E-07                |
| TraesCS5D02G342700.1       | Putrescine<br>hydroxycinnamoyltransferase-<br>like          | 23.7                        | 4.29895E-18                | <b>32.8</b>   | 1.45818E-21                |
| TraesCS5D02G342800.1       | Putrescine<br>hydroxycinnamoyltransferase-<br>like          | <b>7.7</b>                  | 0.001121899                | 5.2   | 0.00419115                 |
| TraesCS6A02G232200.1       | Hydroxycinnamoyltransferase<br>2                            | 2.3                         | 2.70082E-06                | <b>3.4</b>  | 3.16721E-18                |
| TraesCS6D02G203000.1       | Hydroxycinnamoyltransferase<br>2                            | 2.3                         | 1.09564E-06                | <b>3.0</b>  | 2.0977E-20                 |
| TraesCS7B02G054100.1       | Hydroxycinnamoyltransferase<br>4-like                       | 11.1                        | 8.95283E-06                | <b>18.7</b>   | 9.62438E-19                |
| TraesCS5D02G488800.2       | Caffeic acid<br>methyltransferase                           | O- <b>1441.4</b>            | 1.33006E-13                | 981.7   | 1.65969E-13                |
| TraesCS5D02G488900.1       | Caffeic acid<br>methyltransferase                           | O- <b>2195.4</b>            | 8.55349E-16                | 951.1   | 1.04936E-13                |
| TraesCS7A02G333900.1       | Caffeic acid<br>methyltransferase                           | O- 2.1                      | 5.79495E-07                | <b>2.5</b>  | 7.92317E-19                |
| TraesCS7B02G245500.1       | Caffeic acid<br>methyltransferase                           | O- 2.4                      | 9.23752E-09                | <b>3.1</b>  | 1.3994E-30                 |
| TraesCS7D02G341500.1       | Caffeic acid<br>methyltransferase                           | O- 2.4                      | 3.07016E-08                | <b>3.2</b>  | 1.19716E-47                |
| TraesCS7A02G127600.1       | Caffeoyl-CoA<br>methyltransferase 1                         | O- 6.6                      | 6.98663E-25                | <b>10.3</b>   | 2.6911E-125                |
| TraesCS7B02G027200.1       | Caffeoyl-CoA<br>methyltransferase 1                         | O- 4.4                      | 5.35854E-16                | <b>5.7</b>  | 2.81754E-62                |
| TraesCS7B02G136100.1       | Caffeoyl-CoA<br>methyltransferase 2                         | O- 18.6                     | 1.01761E-13                | <b>21.4</b>   | 7.63642E-27                |
| TraesCS7D02G126000.1       | Caffeoyl-CoA<br>methyltransferase 1                         | O- 4.4                      | 2.14511E-17                | <b>6.6</b>  | 2.57338E-45                |
| TraesCS2D02G489700.1       | WRKY45-like transcription<br>factor                         | 3.8                         | 2.87185E-08                | <b>5.9</b>  | 1.13067E-27                |

<sup>1</sup> TraesCS5B02G336600 (Traes\_5AL\_E23B0E6C4) and TraesCS5D02G488900 (Traes\_5DL\_7F0CD0F79) were *F. graminearum*-induced only in resistant recombinant inbred line carrying resistant allele of *QTL-Fhb2* in <sup>[32]</sup>; TraesCS2D02G489700.1 codes for TaWRKY70 <sup>[99]</sup>.

<sup>2</sup> Values that are higher in one genotype compared to the other are highlighted in bold.

**Table S1** The most enriched 50 molecular functions associated with *Fusarium graminearum*-upregulated transcripts in the TaNACL-D1-overexpressing cv. Fielder and the wild type at one day post-inoculation.

| Molecular function <sup>2</sup>            | WT <sup>1</sup>  |  |   |                               | OE <sup>1</sup>  |  |   |                               |
|--|------------------|--|---|-------------------------------|------------------|--|---|-------------------------------|
|  | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> |
| Catalytic activity                         | 1.18371E-88      | 2985   | 27108   | 0.11                          | 1.08078E-91      | 3648   | 27130   | 0.13                          |
| Ion binding                                | 1.12614E-58      | 1972   | 17281   | 0.11                          | 4.61019E-54      | 2369   | 17301   | 0.14                          |
| Organic cyclic compound binding            | 0.001321893      | 1845   | 20649   | 0.09                          | 0.00069574       | 2298   | 20624   | 0.11                          |
| Heterocyclic compound binding              | 0.001199349      | 1843   | 20615   | 0.09                          | 0.000527387      | 2297   | 20591   | 0.11                          |
| Transferase activity                       | 5.61145E-58      | 1476   | 12058   | 0.12                          | 5.17446E-67      | 1818   | 12052   | 0.15                          |
| Small molecule binding                     | 2.58302E-35      | 1228   | 10585   | 0.12                          | 3.02048E-34      | 1480   | 10588   | 0.14                          |
| Anion binding                              | 3.78911E-40      | 1214   | 10185   | 0.12                          | 5.49084E-39      | 1460   | 10194   | 0.14                          |
| Nucleoside phosphate binding               | 1.54706E-29      | 1146   | 10050   | 0.11                          | 9.10189E-29      | 1386   | 10063   | 0.14                          |
| Nucleotide binding                         | 1.54706E-29      | 1146   | 10050   | 0.11                          | 9.10189E-29      | 1386   | 10063   | 0.14                          |
| Carbohydrate derivative binding            | 5.78248E-33      | 1085   | 9222  | 0.12                          | 1.77029E-32      | 1309   | 9227  | 0.14                          |
| Ribonucleotide binding                     | 4.20887E-29      | 1057   | 9142  | 0.12                          | 3.54818E-29      | 1281   | 9150  | 0.14                          |
| Purine nucleotide binding                  | 6.41862E-25      | 1026   | 9059  | 0.11                          | 2.06094E-24      | 1241   | 9057  | 0.14                          |
| Purine ribonucleotide binding              | 1.32576E-25      | 1025   | 9007  | 0.11                          | 1.36574E-24      | 1236   | 9004  | 0.14                          |
| Purine ribonucleoside triphosphate binding | 2.99324E-26      | 1008   | 8791  | 0.11                          | 9.15765E-26      | 1218   | 8789  | 0.14                          |
| Adenyl ribonucleotide binding              | 2.8634E-36       | 1005   | 8233  | 0.12                          | 2.24937E-35      | 1205   | 8230  | 0.15                          |
| Adenyl nucleotide binding                  | 2.09804E-35      | 1005   | 8275  | 0.12                          | 3.8074E-35       | 1209   | 8273  | 0.15                          |
| ATP binding                                | 3.43182E-37      | 988  | 8017  | 0.12                          | 6.44014E-37      | 1187   | 8015  | 0.15                          |

Table S8 continued...

| Molecular function <sup>2</sup>                                 | WT <sup>1</sup>  |  |   |                               | OE <sup>1</sup>  |  |   |                               |
|---|------------------|--|---|-------------------------------|------------------|--|---|-------------------------------|
|   | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> |
| Catalytic activity. acting on a protein                         | 5.15726E-13      | 905  | 8608  | 0.11                          | 5.94669E-17      | 1137   | 8607  | 0.13                          |
| Cation binding  | 1.93858E-13      | 900  | 8518  | 0.11                          | 9.16643E-10      | 1067   | 8527  | 0.13                          |
| Metal ion binding   | 3.65553E-13      | 891  | 8443  | 0.11                          | 2.5835E-09       | 1054   | 8451  | 0.12                          |
| Transferase activity. transferring phosphorus-containing groups | 2.24833E-45      | 764  | 5497  | 0.14                          | 1.63909E-49      | 920  | 5459  | 0.17                          |
| Kinase activity   | 2.5501E-61       | 749  | 4870  | 0.15                          | 1.71939E-68      | 902  | 4836  | 0.19                          |
| Oxidoreductase activity   | 1.17401E-63      | 730  | 4637  | 0.16                          | 2.06186E-61      | 854  | 4652  | 0.18                          |
| Phosphotransferase activity. alcohol group as acceptor          | 6.7187E-67       | 693  | 4234  | 0.16                          | 7.81509E-76      | 837  | 4216  | 0.20                          |
| Protein kinase activity   | 2.70315E-81      | 666  | 3692  | 0.18                          | 1.44822E-94      | 809  | 3685  | 0.22                          |
| Protein serine/threonine kinase activity                        | 2.11677E-82      | 515  | 2489  | 0.21                          | 8.02568E-92      | 613  | 2483  | 0.25                          |
| Transition metal ion binding                                    | 2.51674E-09      | 358  | 3082  | 0.12                          | 3.83715E-08      | 427  | 3099  | 0.14                          |
| Transporter activity  | 8.94135E-07      | 358  | 3254  | 0.11                          | 0.000155799      | 416  | 3252  | 0.13                          |
| Transmembrane transporter activity                              | 3.24984E-06      | 341  | 3117  | 0.11                          | 5.57135E-05      | 404  | 3113  | 0.13                          |
| Heme binding  | 7.37426E-72      | 281  | 1013  | 0.28                          | 9.82412E-72      | 318  | 1019  | 0.31                          |
| Tetrapyrrole binding  | 8.11297E-62      | 281  | 1121  | 0.25                          | 1.03041E-60      | 318  | 1125  | 0.28                          |
| Transcription regulator activity                                | 0.021726873      | 250  | 2497  | 0.10                          | 8.47978E-08      | 355  | 2519  | 0.14                          |
| DNA-binding transcription factor activity                       | 1.1892E-05       | 240  | 2105  | 0.11                          | 1.6038E-13       | 337  | 2125  | 0.16                          |
| Glycosyltransferase activity                                    | 1.43463E-10      | 237  | 1817  | 0.13                          | 1.59886E-14      | 302  | 1822  | 0.17                          |
| Carbohydrate binding  | 6.80016E-58      | 227  | 821   | 0.28                          | 2.34363E-60      | 262  | 828   | 0.32                          |
| Monooxygenase activity  | 3.39211E-47      | 217  | 870   | 0.25                          | 1.34779E-43      | 241  | 877   | 0.27                          |

Table S8 continued...

| Molecular function <sup>2</sup>   | WT <sup>1</sup>  |  |   |                               | OE <sup>1</sup>  |  |   |                               |
|---|------------------|--|---|-------------------------------|------------------|--|---|-------------------------------|
|   | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> | FDR <sup>3</sup> | Number of <i>F. graminearum</i> -regulated transcripts | Number of transcripts in the reference <sup>4</sup> | Transcript ratio <sup>5</sup> |
| Oxidoreductase activity. acting on paired donors. with incorporation or reduction of molecular oxygen | 8.49149E-39      | 209  | 922   | 0.23                          | 1.15248E-35      | 234  | 931   | 0.25                          |
| Lyase activity  | 2.93857E-21      | 209  | 1231  | 0.17                          | 1.0577E-17       | 232  | 1223  | 0.19                          |
| Active transmembrane transporter activity   | 8.64757E-16      | 207  | 1357  | 0.15                          | 4.56094E-14      | 239  | 1365  | 0.18                          |
| Iron ion binding  | 2.04436E-36      | 204  | 920   | 0.22                          | 1.6274E-35       | 234  | 933   | 0.25                          |
| UDP-glycosyltransferase activity  | 1.88562E-23      | 171  | 883   | 0.19                          | 1.3863E-26       | 204  | 873   | 0.23                          |
| Acyltransferase activity  | 2.24592E-08      | 168  | 1252  | 0.13                          | 5.94282E-08      | 198  | 1249  | 0.16                          |
| Acyltransferase activity. transferring groups other than amino-acyl groups                            | 1.47284E-10      | 165  | 1145  | 0.14                          | 7.07789E-11      | 196  | 1139  | 0.17                          |
| Hydrolase activity. acting on glycosyl bonds  | 0.00255705       | 150  | 1338  | 0.11                          | 0.012515273      | 177  | 1344  | 0.13                          |
| Calcium ion binding   | 1.63308E-14      | 145  | 860   | 0.17                          | 1.02107E-13      | 167  | 859   | 0.19                          |
| Transferase activity. transferring alkyl or aryl (other than methyl) groups                           | 1.37113E-36      | 140  | 496   | 0.28                          | 4.0074E-39       | 164  | 506   | 0.32                          |
| Hydrolase activity. Hydrolyzing O-glycosyl compounds  | 0.005549895      | 131  | 1165  | 0.11                          | 0.018769882      | 155  | 1171  | 0.13                          |
| Protein threonine kinase activity   | 8.86198E-14      | 125  | 713   | 0.18                          | 2.54676E-13      | 145  | 717   | 0.20                          |
| Protein serine kinase activity  | 8.86198E-14      | 125  | 713   | 0.18                          | 2.54676E-13      | 145  | 717   | 0.20                          |
| Glutathione transferase activity  | 7.50998E-62      | 121  | 242   | 0.50                          | 9.12866E-68      | 140  | 250   | 0.56                          |

<sup>1</sup> OE = TaNACL-D1-overexpressing cv. Fielder; WT = wild type.

<sup>2</sup> Due to many upregulated molecular processes (>100 in both genotypes), only the top 50 of those that were the most enriched are listed in the table.

<sup>3</sup> FDR = False discovery rate (Fischer's exact test, FDR <0.05).

<sup>4</sup>Reference = The number of all expressed transcripts associated with the molecular function in the corresponding genotype.

<sup>5</sup>Transcript ratio = The number of *F. graminearum*-regulated transcripts associated with the molecular function relative to the total number of expressed transcripts associated with the same molecular function in the corresponding genotype.



**Table S2** The transcripts that were significantly *Fusarium*-regulated in the TaNACL-D1-overexpressing cv. Fielder (OE) but not in the wild type (WT), and that were present at significantly higher or lower levels in the pathogen-treated OE versus WT, and that had functionally characterised *Arabidopsis thaliana* top BLAST hits.

| Transcript ID        | Arabidopsis top BLAST hit | E-value     | % Identity | Proven function of the Arabidopsis homolog <sup>a</sup>  | Reference  |
|----------------------|---------------------------|-------------|------------|--|------------|
| TraesCS3D02G340500.1 | AT5G50260.1               | 3.0339E-170 | 74         | Tapetal programmed cell death, pollen development, secondary cell wall thickening during xylem development, resistance against biotrophic pathogen   | [69–71]    |
| TraesCS3B02G378600.1 |                           |             |            |  |            |
| TraesCS3D02G342100.1 |                           |             |            |  |            |
| TraesCS3B02G380400.1 |                           |             |            |  |            |
| TraesCS3D02G411200.5 | AT3G43920.2               | 0           | 59         | Positively regulates flowering time; Inactivated by the viral silencing suppressor protein 2b of the cucumber mosaic virus   | [100, 101] |
| TraesCS6B02G176700.1 | AT5G25060.1               | 0           | 77         | phytochrome B signalling   | [102]      |
| TraesCS4B02G131700.2 | AT5G13750.1               | 8.1704E-163 | 65         | Regulates stomatal movements and polar auxin transport by modulating potassium and proton fluxes   | [103]      |
| TraesCS2D02G294600.1 | AT5G04820.1               | 1.00294E-29 | 51         | A negative regulator of floral initiation  | [104]      |
| TraesCS6A02G109100.2 | AT4G31120.1               | 0           | 82         | A positive regulator of floral initiation  | [105]      |
| TraesCS5D02G383900.3 | AT4G21710.1               | 0           | 96         | Development of embryo  | [106]      |
| TraesCS1B02G431800.5 | AT5G04430.1               | 1.04799E-65 | 61         | Negative regulator of tomato mosaic virus multiplication   | [34]       |
| TraesCS2B02G556100.3 | AT1G50840.1               | 0           | 79         | DNA replication  | [107]      |
| TraesCS2D02G396500.2 | AT1G12520.1               | 1.9134E-119 | 80         | Negative regulator of heat stress-responsive genes and thermotolerance   | [108]      |
| TraesCS7B02G322500.2 | AT2G28880.1               | 0           | 72         | Biosynthesis of PABA   | [109]      |
| TraesCS2B02G603800.1 | AT5G04810.1               | 0           | 81         | Embryo development; acts in the plastid nucleoids as the factor responsible of <i>rps12</i> intron 1 trans-splicing and, indirectly, in the assembly of 70S ribosomes and plastid translation. | [110, 111] |
| TraesCS3B02G367800.1 | AT3G45100.3               | 8.291E-158  | 89         | Involved in the biosynthesis of GPI; <i>loss of function</i> reduced pollen germination and tube growth, which are associated with abnormal callose deposition.                                | [112]      |
| TraesCS5D02G112200.1 | AT1G80570.3               | 7.31835E-64 | 53         | Regulates the proteolysis of cell cycle transcription factors and connects auxin signalling with cell division; bound and activated by auxin.  | [113]      |

**Table S9** continued...

| Transcript ID        | Arabidopsis top BLAST hit | E-value     | % Identity | Proven function of the Arabidopsis homolog <sup>a</sup>   | Reference  |
|----------------------|---------------------------|-------------|------------|---|------------|
| TraesCS4A02G151000.1 | AT1G78300.1               | 1.72344E-95 | 70         | Interacts with THRUMIN1 in light-dependent chloroplast movements; Interacts with ABF3 in the nucleus; negative regulator of EOL2 (ethylene biosynthesis) protein stability; positively regulates ACS (ethylene biosynthesis) protein stability via the 26S proteasome-dependent pathway | [114–116]  |
| TraesCS1B02G288600.2 | AT1G32540.1               | 9.60995E-49 | 90         | Positive regulator of cell death  | [73]       |
| TraesCS7D02G103000.1 | AT1G67730.1               | 2.00125E-59 | 65         | Required for the elongation of fatty acids precursors of sphingolipids, triacylglycerols, cuticular waxes and suberin; Embryo development; Interacts with a cell death suppressor and positive regulator of stress resistance BI-1.   | [117, 118] |
| TraesCS2D02G043300.1 | AT5G54650.2               | 1.3648E-163 | 69         | Promotes actin polymerization; pollen germination   | [119, 120] |

<sup>a</sup> PABA = para-aminobenzoic acid; RSP12 = ribosomal protein S 12; GPI = glycosylphosphatidylinositol; ABF3 = abscisic acid-insensitive 5-like protein 6 ; EOL2 = ETO-1 like 2; ACS = acetyl-coA synthetase; BI-1 = Bax inhibitor 1.

**Table S10** The transcripts that were significantly *Fusarium*-regulated in the TaNACL-D1-overexpressing cv. Fielder (OE) but not in the wild type (WT), and that were present at significantly higher or lower levels in the pathogen-treated OE versus WT, and that had functionally characterised rice (*Oryza sativa*) top BLAST hits.

| Transcript ID                                   | Rice top BLAST hit | E-value  | % Identity | Proven function of rice homolog <sup>a</sup>   | Reference  |
|---|--------------------|----------|------------|--|------------|
| TraesCS3D02G34050 0.1;<br>TraesCS3B02G37860 0.1 | Os08t055690 0-01   | 0        | 89         | Negatively regulates stress-induced cell death   | [72]       |
| TraesCS3D02G34210 0.1;<br>TraesCS3B02G38040 0.1 | Os08t055690 0-02   | 0        | 89         | Negatively regulates stress-induced cell death   | [72]       |
| TraesCS3D02G41120 0.5                           | Os01t090920 0-01   | 0        | 79         | Directly targets genes involved in gibberellin and brassinosteroid homeostasis; Silencing caused dwarfism, larger flag leaf angle, and fewer secondary branches; phased small RNA biogenesis                   | [121, 122] |
| TraesCS4B02G13170 0.2                           | Os12t013310 0-02   | 0        | 72         | Involved in sequestering a phytosiderophore DMA into the vacuoles. Knockout enhanced accumulation of Fe and Zn in polished rice grains through DMA-increased solubilization of Fe and Zn deposited in the node | [123]      |
| TraesCS2B02G55610 0.3                           | Os08t017530 0-01   | 0        | 75         | Plastidial DNA replication and repair  | [124]      |
| TraesCS2B02G60380 0.1                           | Os04t068450 0-01   | 0        | 95         | Chloroplast biogenesis in rice under cold stress; intron splicing of rpl2 and rps12 in chloroplasts  | [125]      |
| TraesCS4A02G15100 0.1                           | Os11t054690 0-01   | 2.1E-113 | 78         | Interacts with BZR1 (brassinosteroid signal transduction in rice); Interacts with ABI5 (involved in ABA signalling, growth, and abiotic stress responses)  | [126, 127] |
| TraesCS1B02G28860 0.2                           | Os08t015950 0-01   | 1E-54    | 96         | Negative regulator of PCD and defence against virulent blast fungus; and positive regulator of callus differentiation and tolerance to fumonisin 1   | [74]       |
| TraesCS2D02G17620 0.3                           | Os07t061600 0-01   | 0        | 78         | Silencing negatively affected grain size due to reduction of cell number and cell size, and increased stem thickness and number of vascular bundles  | [128, 129] |
| TraesCS7D02G00130 0.1                           | Os12t024770 0-01   | 5.08E-70 | 57         | Quantitative broad-spectrum resistance against different pathogens including bacteria, oomycetes, and fungi  | [130]      |

<sup>a</sup> DMA = 29-deoxymugineic acid; BZR1 = brassinazole-resistant 1; ABI5 = ABA insensitive 5; ABA = abscisic acid; PCD = programmed cell death; Fe = iron; Zn = zinc.

**Table S11** The transcripts that were present at significantly higher or lower levels in the non-treated (day zero) *TaNAACL-D1*-overexpressing cv. Fielder as compared to the wild type, and that were associated with development-related biological processes.

| Transcript ID        | Fold change | Description  | Biological processes  |
|----------------------|-------------|--|---|
| TraesCS2D02G387600.1 | 0.01        | Probable metal-nicotianamine transporter YSL9        | Response to iron ion; siderophore-dependent iron import into cell; seed development; transmembrane transport  |
| TraesCS3B02G054400.1 | 0.13        | E3 ubiquitin-protein ligase SINA-like 10             | Ubiquitin-dependent protein catabolic process; multicellular organism development; protein ubiquitination   |
| TraesCS6A02G312500.2 | 0.01        | Probable histidine kinase 6                          | Phosphorylated signal transduction system; protein dephosphorylation; osmosensory signalling pathway; sulfate transport; nucleoside metabolic process; response to water deprivation; cytokinin-activated signalling pathway; regulation of seed germination; embryonic root morphogenesis; cellular response to phosphate starvation; peptidyl-histidine phosphorylation; carbohydrate homeostasis; defence response to bacterium; regulation of meristem development; regulation of shoot system development; cellular response to sucrose stimulus |
| TraesCS6B02G183600.1 | 0.02        | Succinate-semialdehyde dehydrogenase , mitochondrial | Cellular aldehyde metabolic process; glutamate decarboxylation to succinate; response to heat; response to cold; response to light stimulus; gamma-aminobutyric acid catabolic process; adaxial/abaxial axis specification; maintenance of shoot apical meristem identity; cotyledon development; reactive oxygen species metabolic process; response to salt   |
| TraesCS7A02G053900.1 | 0.50        | Predicted protein                                    | Embryo development ending in seed dormancy  |
| TraesCS7A02G371200.3 | 0.02        | Protein argonaute PNH1                               | Stem cell population maintenance; gene silencing by RNA; leaf development   |
| TraesCS7B02G021100.1 | <0.01       | MADS-box transcription factor 5                      | Flower development; cell differentiation; positive regulation of transcription by RNA polymerase II   |
| TraesCS1D02G162600.2 | 128         | Protein YABBY 3                                      | Polarity specification of adaxial/abaxial axis; fruit development; abaxial cell fate specification; regulation of shoot apical meristem development; regulation of leaf development   |
| TraesCS3B02G224600.3 | 128         | DNA replication licensing factor MCM4                | Double-strand break repair via break-induced replication; pre-replicative complex assembly involved in nuclear cell cycle DNA replication; DNA unwinding involved in DNA replication; DNA strand elongation involved in DNA replication; pollen development; mitotic DNA replication initiation   |
| TraesCS5D02G066200.1 | 2           | Probable DNA helicase MCM8                           | Double-strand break repair via homologous recombination; male meiotic nuclear division; female meiotic nuclear division; pollen development; DNA duplex unwinding   |

**Table S12** The transcripts that were present at significantly higher or lower levels in the TaNACL-D1-overexpressing cv. Fielder as compared to the wild type irrespective of the treatment and that had functionally characterised *Arabidopsis* top BLAST hits.

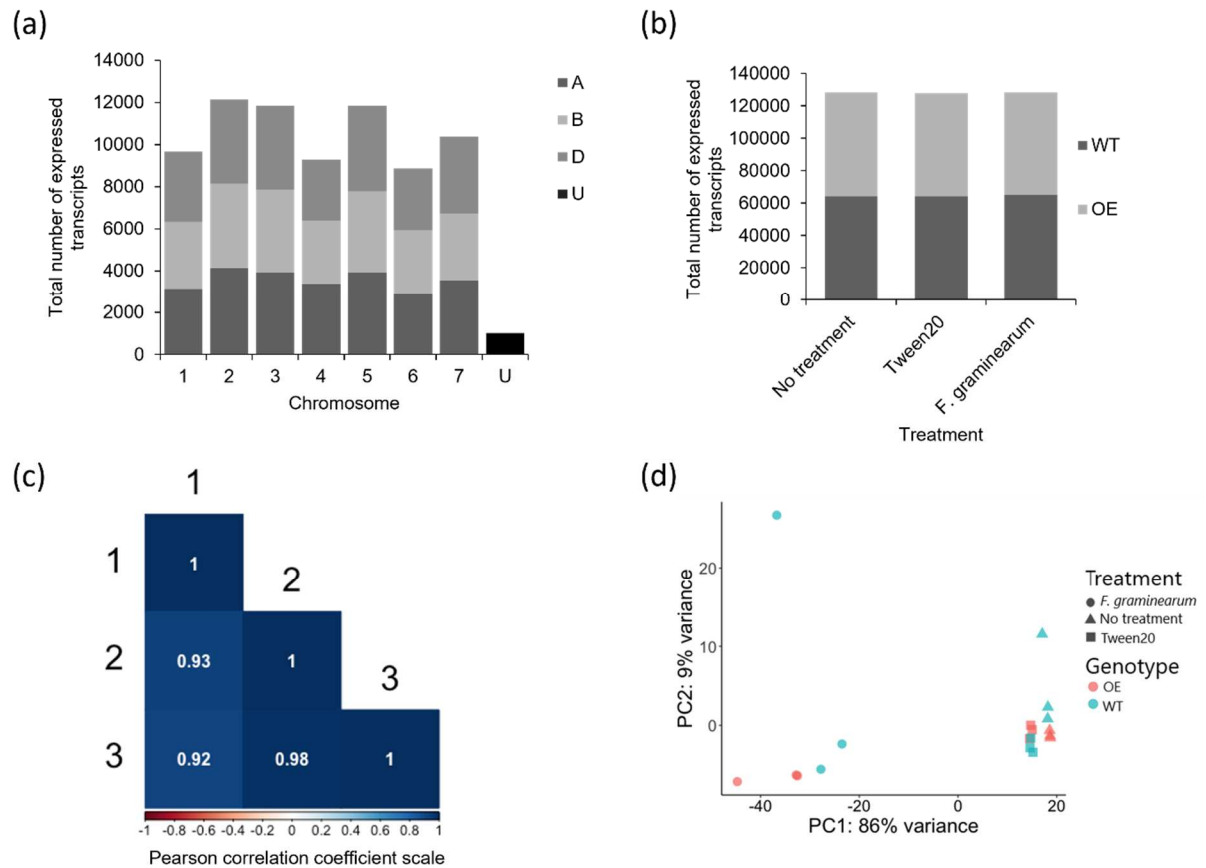
| Transcript ID        | Arabidopsis top BLAST hit | E-value      | % Identity | Proven function of Arabidopsis homolog <sup>1</sup>   | Reference     |
|----------------------|---------------------------|--------------|------------|---|---------------|
| TraesCS5D02G111300.1 | AT1G02250.1               | 2.39712E-7   | 42         | Xylem development   | [36]          |
| TraesCS5A02G259000.1 | AT1G06390.2               | 8.71406E-25  | 91         | Brassinosteroid signalling and salt tolerance   | [41, 131]     |
| TraesCS1B02G005000.1 | AT1G09730.2               | 7.83879E-4   | 50         | Floral initiation, flower development, embryo development and gametogenesis, ABA signalling | [37, 38, 132] |
| TraesCS5B02G104200.1 | AT1G02250.1               | 4.1898E-9    | 39         | Xylem development   | [36]          |
| TraesCS4A02G469500.1 | AT2G03200.1               | 3.82748E-75  | 51         | Primary and lateral root development  | [39]          |
| TraesCS2B02G042700.2 | AT3G14460.1               | 9.60435E-77  | 56         | Defence against biotrophic and hemibiotrophic plant pathogens                               | [44]          |
| TraesCS3D02G349200.1 | AT3G53520.1               | 0.0          | 80         | Biosynthesis of UDP-xylose  | [42]          |
| TraesCS6A02G330100.1 | AT1G44800.1               | 3.85612E-125 | 70         | Amino acid homeostasis in siliques  | [45]          |
| TraesCS1B02G361800.1 | AT1G06390.2               | 8.71406E-25  | 91         | Brassinosteroid signalling and salt tolerance   | [41, 131]     |
| TraesCS7B02G375300.1 | AT1G79550.2               | 0.0          | 91         | Glycolysis  | [43]          |

<sup>1</sup> ABA = abscisic acid; UDP = uridine diphosphate.

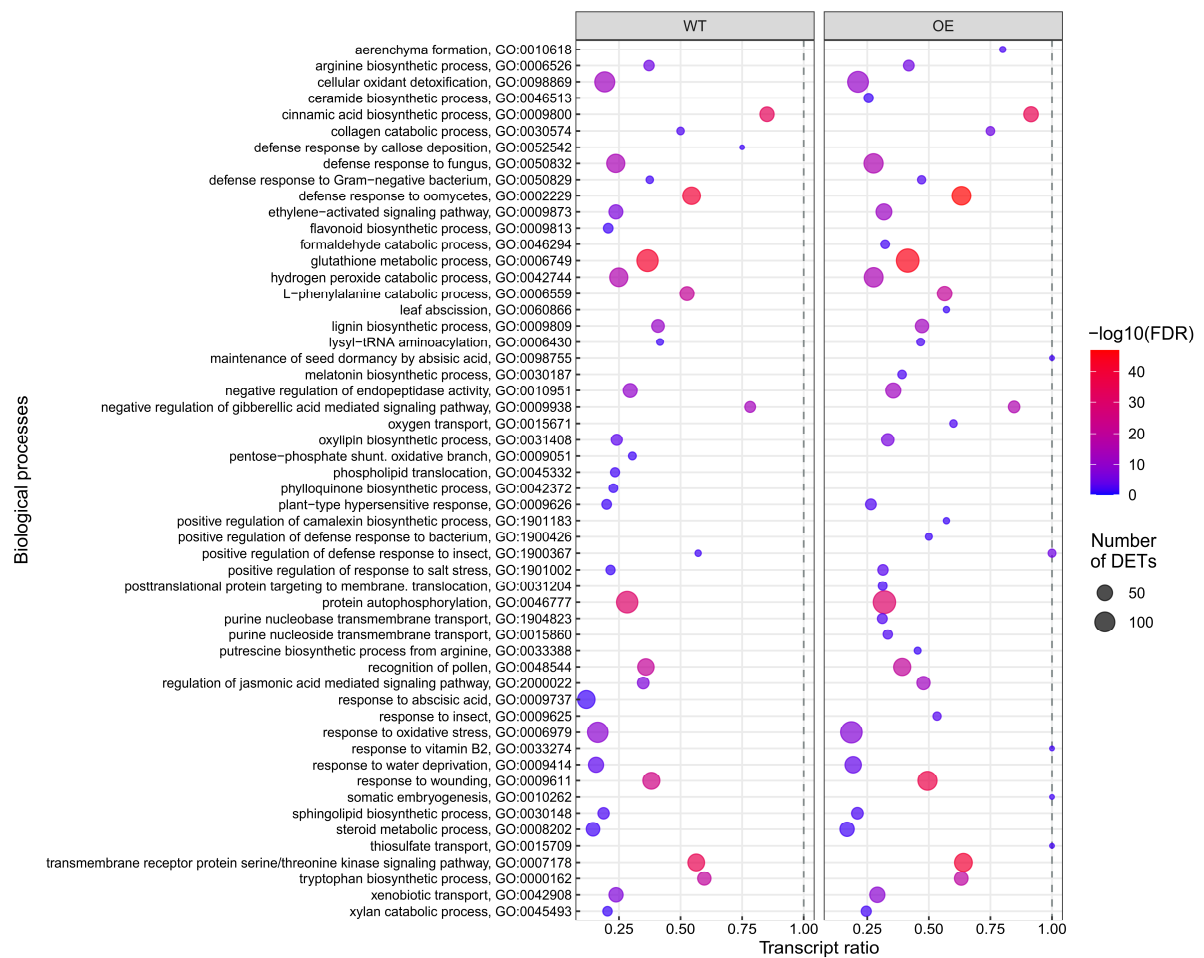
**Table S13** The transcripts that were present at significantly higher or lower levels in the TaNACL-D1-overexpressing cv. Fielder as compared to the wild type irrespective of the treatment and that had functionally characterised rice (*Oryza sativa*) top BLAST hits.

| Transcript ID        | Rice top BLAST hit | E-value     | % Identity | Proven function of rice homolog                       | Reference |
|----------------------|--------------------|-------------|------------|---|-----------|
| TraesCS5A02G259000.1 | Os05t0207500-01    | 2.45862E-26 | 89         | Negative regulator of brassinosteroid (BR) signalling | [40]      |
| TraesCS4A02G156500.1 | Os03t0397400-01    | 0.0         | 95         | Ca <sup>2+</sup> transport                            | [46]      |
| TraesCS1B02G361800.1 | Os05t0207500-01    | 2.13037E-26 | 89         | Negative regulator of brassinosteroid signalling      | [40]      |

## SUPPLEMENTAL FIGURES

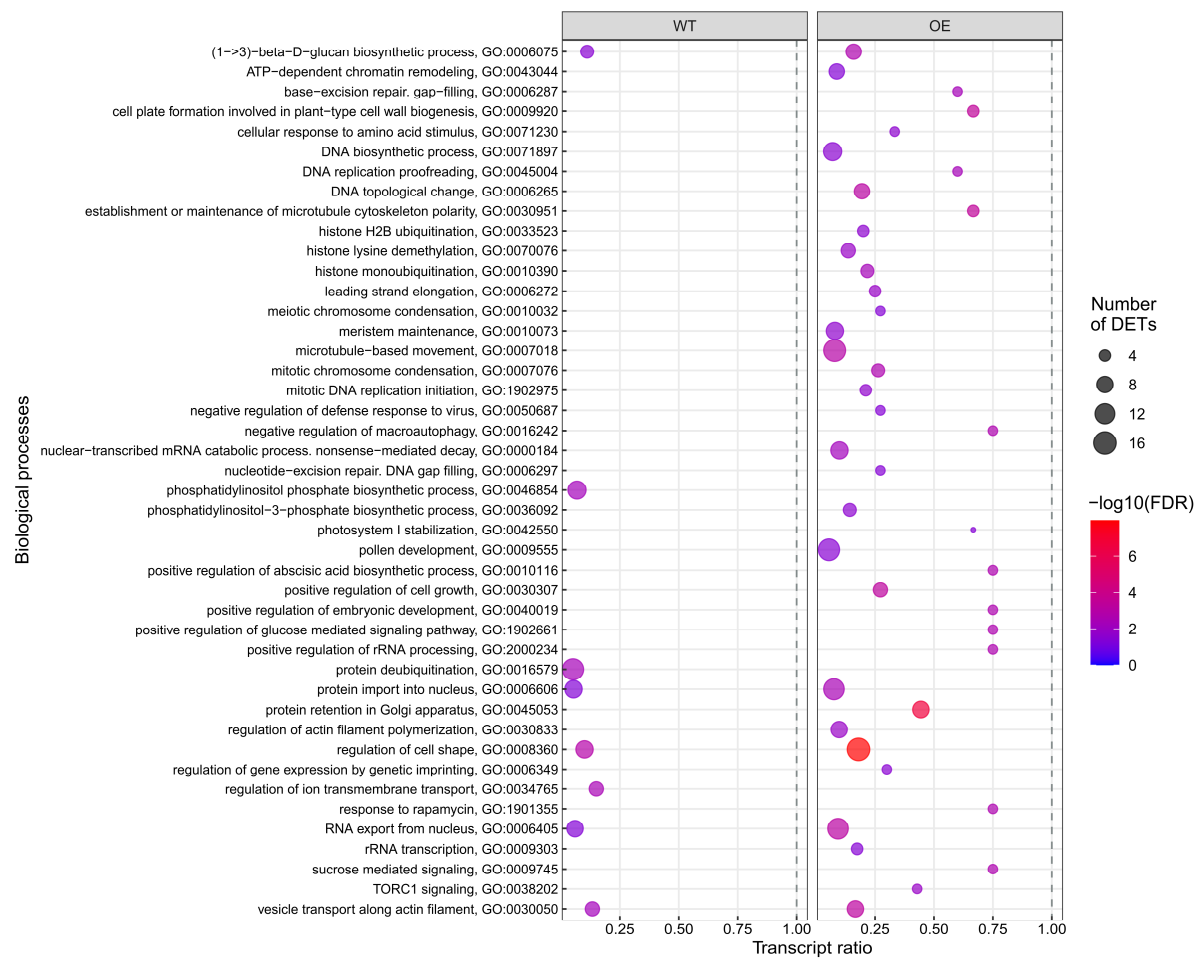


**Figure S1** Quality assessment of RNA transcriptomes from 18 sequenced samples: **(a)** distribution of expressed transcripts across wheat subgenomes (A, B, D) and chromosomes (1-7), including an unknown subgenome/chromosome U; **(b)** number of expressed transcripts within each treatment x genotype combination; **(c)** the Pearson's correlation of expressed transcripts between three trials. The correlation coefficient within each box represents each pairwise correlation; and **(d)** principal component (PC) plot of variance-stabilised-transformed read counts across 18 samples. Circles, triangles, and squares indicate *Fusarium graminearum* treatment, no-treatment control (day zero) and Tween 20 treatment (mock), respectively. Colours refer to the genotypes: OE (overexpressing line) and WT (wild type).

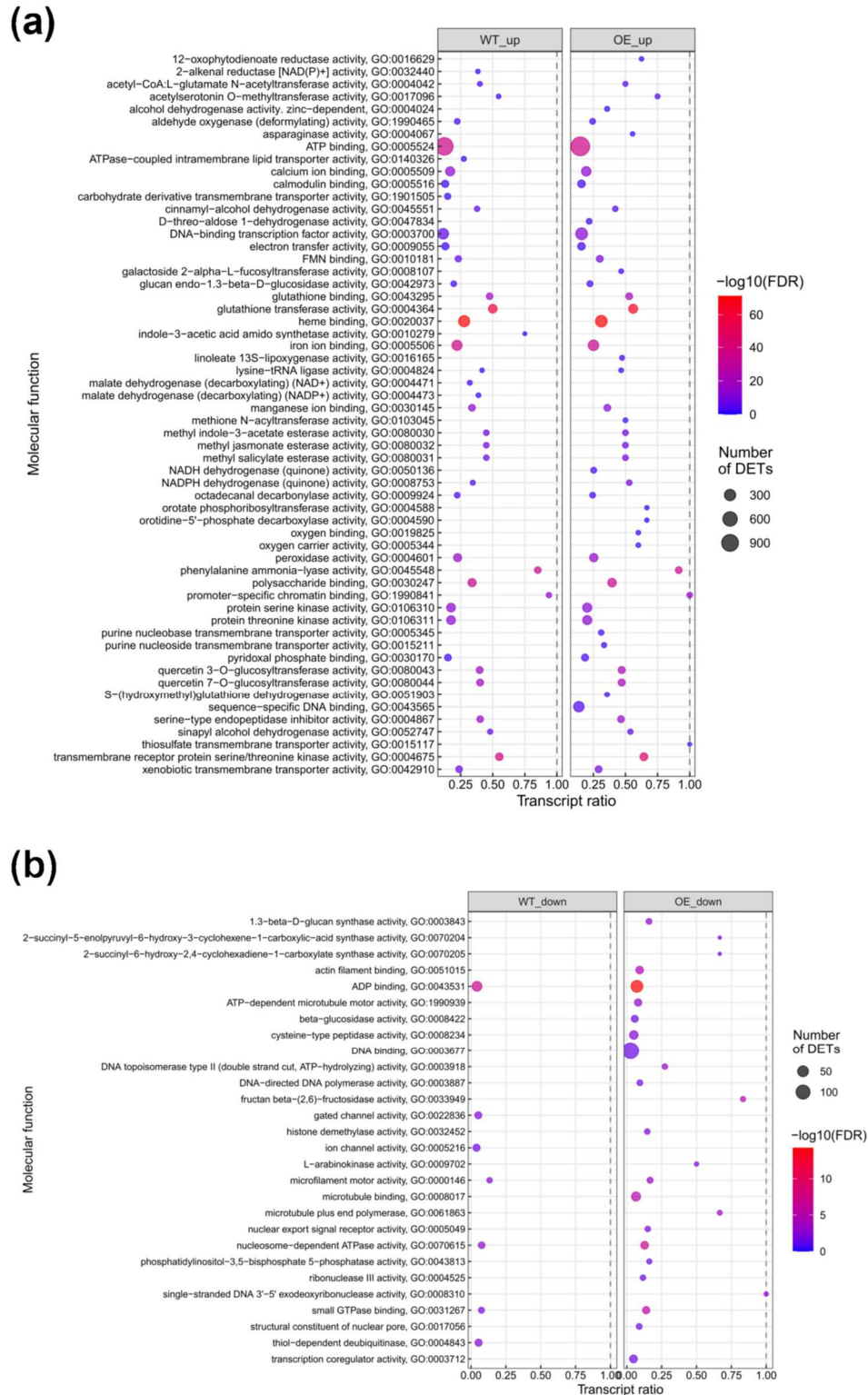


**Figure S2** Descent biological processes associated with *Fusarium graminearum*-upregulated transcripts in the TaNACL-D1-overexpressing (OE) wheat cv. Fielder and the WT at one day post-inoculation. The coloured scale on the right represents negative logarithmic scale with a base 10 of the false discovery rate (FDR) values for each of these processes. Abbreviation: DETs, differentially expressed transcripts.

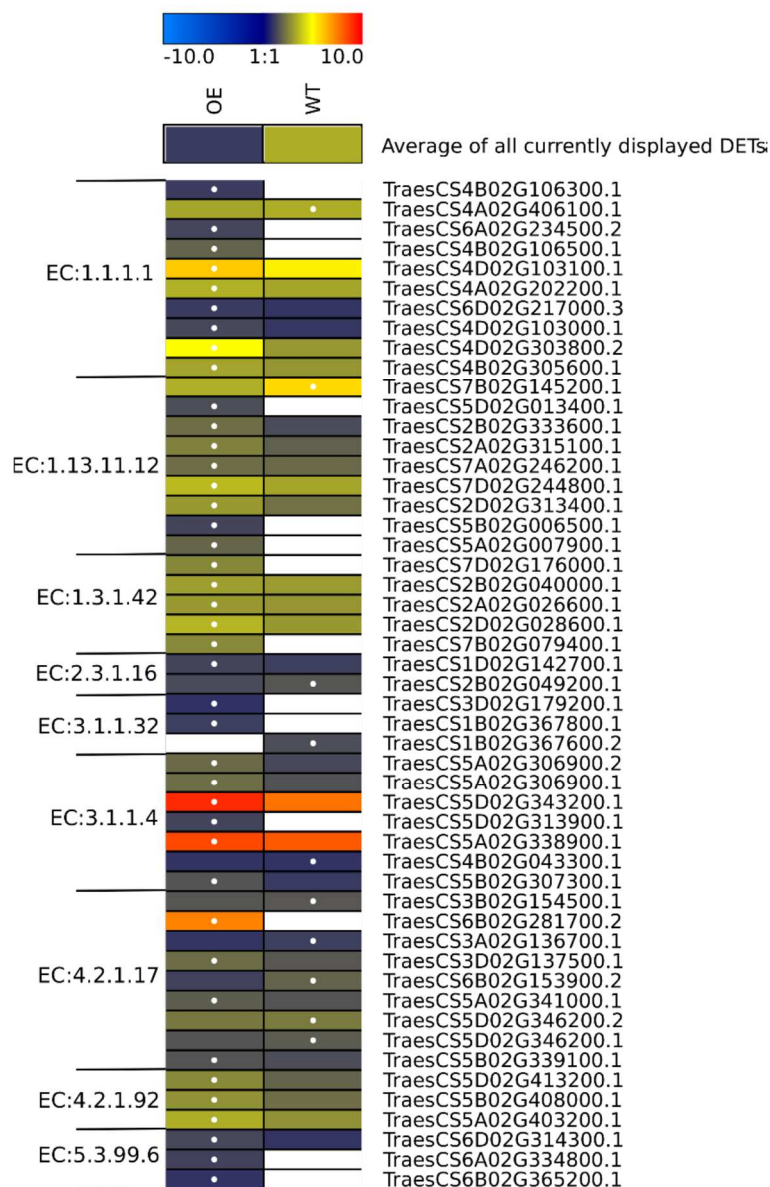




**Figure S3** Descent biological processes associated with *Fusarium graminearum*-downregulated transcripts in the TaNACL-D1-overexpressing (OE) wheat cv. Fielder and the wild type (WT) at one day post-inoculation. The coloured scale on the right represents negative logarithmic scale with a base 10 of the false discovery rate (FDR) values for each of these processes. Abbreviation explanation: DETs = differentially expressed transcripts.



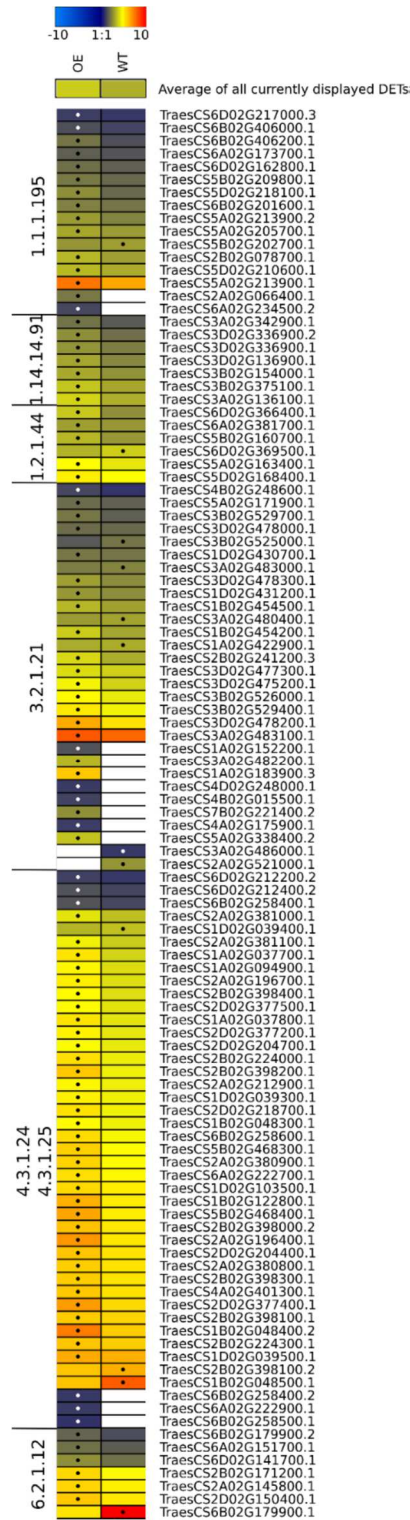
**Figure S4** Descent molecular functions significantly enriched with associated: **(a)** *Fusarium graminearum*-upregulated; and **(b)** *F. graminearum*-downregulated transcripts in the TaNACL-D1-overexpressing cv. Fielder (OE) and the wild type (WT). The coloured scale on the right represents negative logarithmic scale with a base 10 of the false discovery rate (FDR) values for each of these molecular functions. Abbreviations explanation: up = upregulated; down = downregulated; DETs = differentially expressed transcripts.



**Figure S5** The heatmap of the *F. graminearum*-upregulated transcripts associated with alpha-linolenic acid metabolism at one day post-inoculation in the TaNACL-D1-overexpressing cv. Fielder (OE) and the wild type (WT). Enzyme codes: 1.1.1.1 = Alcohol dehydrogenase; 1.3.11.12 = linoleate 13S-lipoxygenase; 1.3.1.42 = 12-oxophytodienoate reductase; 2.3.1.16 = acetyl-CoA C-acyltransferase; 3.1.1.32 = phospholipase A1; 3.1.1.4 = phospholipase A2; 4.2.1.17 = enoyl-CoA hydratase; 4.2.1.92 = hydroperoxide dehydratase; 5.3.99.6 = allene-oxide cyclase. The coloured scale on the top represents log2 fold change. The white fields represent transcripts that are not regulated by the fungus. A dot within a coloured field indicates that the expression of the transcript in that genotype differed as compared to the other genotype. Abbreviation: DETs, differentially expressed transcripts.

**Figure S6** The map of the alpha-Linolenic acid metabolism KEGG pathway (map00592) [133]. The coloured enzyme codes are associated with the *F. graminearum*-upregulated transcripts at one day post-inoculation in both the TaNACL-D1-overexpressing cv. Fielder and the wild type. Enzyme codes: 1.1.1.1 = alcohol dehydrogenase; 1.3.11.12 = linoleate 13S-lipoxygenase; 1.3.1.42 = 12-oxophytodienoate reductase; 2.3.1.16 = acetyl-CoA C-acyltransferase; 3.1.1.32 = phospholipase A1; 3.1.1.4 = phospholipase A2; 4.2.1.17 = enoyl-CoA hydratase; 4.2.1.92 = hydroperoxide dehydratase; 5.3.99.6 = allene-oxide cyclase.

**Figure S7** The map of the linoleic acid metabolism KEGG pathway (map00591) [133]. The coloured enzyme codes are associated with the *F. graminearum*-upregulated transcripts at one day post inoculation in only the TaNACL-D1-overexpressing cv. Fielder (OE) or in both the OE and the wild type (OE/WT) (highlighted with blue font). Enzyme codes: 1.3.11.12 = linoleate 13S-lipoxygenase; 1.3.11.58 = linoleate 9S-lipoxygenase; 3.1.14 =phospholipase A(2).



**Figure S8** The heatmap of the *F. graminearum*-upregulated transcripts associated with the phenylpropanoid biosynthesis at one day post-inoculation in the *TaNACL-D1*-overexpressing cv. Fielder (OE) and the wild type (WT). Enzyme codes: 1.1.1.195 = cinnamyl alcohol dehydrogenase; 1.4.14.91 = Trans-cinnamate 4-monooxygenase; 1.2.1.44 = Cinnamoyl-CoA reductase; 3.2.1.21 = beta-glucosidase; 4.3.1.24/25 = phenylalanine/tyrosine ammonia-lyase ; 6.2.1.12 = 4-coumarate—CoA ligase. The coloured scale on the top represents log2 fold change. The white fields represent transcripts that are not regulated by the fungus. A dot within a coloured field indicates that the expression of the transcript in that genotype differed as compared to the other genotype. Abbreviation explanation: DETs; differentially expressed transcripts.



