## Supplementary Materials: Virus-Induced Gene Silencing Identifies an Important Role of the TaRSR1 Transcription Factor in Starch Synthesis in Bread Wheat

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Figure S1. cDNA sequences for three copies of TaRSR1 gene. The fragment used to VIGS silencing has been underlined. Base pairs signed with black or blue colors are defined as same bases between two or three copies. White pairs signed with white color are defined as different bases between one copy and the other two copies.


Figure S2. Transcription levels of the TaRSR1 gene in the BSMV-TaRSR1-infected and BSMV-GFP-infected wheat plants at 20, 27 and 31 days after anthesis. Transcription levels were measured by qPCR using GAPDH gene as internal control. Each value is the mean $\pm$ standard deviation of three independent biological replicates. Asterisks indicate significant differences ( $p<0.05$ ).


Figure S3. Cont.


Figure S3. Transcription levels of the 26 starch synthesis-related enzyme genes in the grains of BSMV-TaRSR1-infected and BSMV-GFP-infected plants at 20,27 and 31 days after anthesis. Transcription levels were measured by $q$ PCR using GAPDH gene as internal control. (A-Z), transcription levels of TaAGPS1-a, TaAGPS1-b, TaAGPS2, TaAGPL1, TaAGPL2, TaGBSSI, TaGBSSII, TaSSI, TaSSIIa, TaSSIIb, TaSSIIc, TaSSIIIa, TaSSIIIb, TaSSIV, TaBEI, TaBEIIa, TaBEIIb, TaBEIII, TaISA1, TaISA2, TaISA3, TaPUL, TaPHOL, TaPHOH, TaDPE1, and TaDPE2 genes, respectively. Each value is the mean $\pm$ standard deviation of three independent biological replicates. Asterisks indicate significant differences ( $p<0.05$ ).


Figure S4. Schematic construction of the recombinant barley stripe mosaic virus-wheat starch regulator 1 (BSMV-TaRSR1) vector.


Figure S5. Barley stripe mosaic virus-wheat starch regulator 1 (BSMV-TaRSR1) and barley stripe mosaic virus-green fluorescent protein (BSMV-GFP) virus inoculation under field conditions.

Table S1. Target genes for analyzing transcription levels and their primer sequences.

| Gene Name | Accession <br> Number <br> (Acc. No.) | Forward Primer (5'-3') | Reverse Primer (5'-3') | Amplification <br> Sizes Base <br> Pair (bp) |
| :---: | :---: | :---: | :---: | :---: |
| TaAGPS1-a | X66080 | CCTTCCAAGCGTGAACAA | TTCCGAGAACACTATCATCAAC | 94 |
| TaAGPS1-b | EU586278 | AAGATCCTGATCCCTCCG | CTAGTCCCTGCACCACCT | 140 |
| TaAGPS2 | AY727927 | TAATTCCGAGCGGGACAG | AAATGGTGCCTTGAGTGG | 199 |
| TaAGPL1 | Z21969 | GCCCCTGTTGGAGAGAGCCG | TAGCAGGGTCGTCGATGGCG | 157 |
| TaAGPL2 | DQ406820 | ATTGATGGCAGCCGGGCGTC | TCGGTAAGCCGAGGAGAGTGGT | 136 |
| TaGBSSI | AF286320 | CGGCATGGACGTCAGCGAGT | AGGGGCACCTTCCGGTCCAC | 147 |
| TaGBSSII | AF109395 | TGCATTGGAGGCTCCGAGGGT | GCAGTGTGCCAGTCATTTGCAACG | 102 |
| TaSSI | AJ292521 | GAACTGGGGGCCTCCGAGACA | CCTCCCAGGACGGCTTGTGC | 162 |
| TaSSIIa | AJ269503 | CACGCCGGTGAACGGTGAGA | CGGCGAGGCGACGTTAGCTT | 148 |
| TaSSIIb | EU333947 | GCCAGAAGAGGGCATCGCGT | ATTGTGCCGGTGGCGGAAGG | 189 |
| TaSSIIc | EU307274 | GGTCTCCCGGTTCGTGGGGA | TTGCCTCCCGGTACCCAGCA | 144 |
| TaSSIIIa | AF258608 | CGGTTGACGAAGTCGGGCCT | CCCAGCTTCATCCACTGCACCC | 197 |
| TaSSIIIb | EU333946 | GCAGCGTGGTGCTGGAGGTAG | TGATTGGTCCTGTCCTTGGGTGC | 176 |
| TaSSIV | AY044844 | CGACGAGCAGGGCCTAAGCA | AGCCGAGGTGTCCCAGCTGAA | 124 |
| TaBEI | Y12320 | TGGGTCGATCGGGTTCCTGCAT | ACGTGGAGCGTCAGGCTTCG | 150 |
| TaBEIIa | AF286319 | GCAAGTCCGGCGCAACCTGA | CTCGCGGTTTCTCCCCCACG | 190 |
| TaBEIIb | AY740401 | CGCCTTCCATCGACGGTCCC | TCCCGGTGGTGGCAGAATGC | 212 |
| TaBEIII | JQ346193 | TACGTTGACAAGGATGCGCT | CTAATCCGCCTTGGGTGGTT | 139 |
| TaISA1 | AF548380 | AGTACCGGGACATTGTGCGCC | GCCAAGGTTTCCTTCCTCCTGCC | 117 |
| TaISA2 | JX473824 | GCCACGTCTGGCACGTCTCG | TGGCAGCAACCAGGTCACCG | 132 |
| TaISA3 | JN412069 | CCTTGGGATTGTGGAGGTCTTT | CATGATGAGGCTTCCGCTGGTT | 193 |
| TaPUL | EF137375 | CCCTCCTCAGTCCCAGGTGT | TGCGGACACACATTGAAGACCG | 123 |
| TaPHOL | EU595762 | TGGGGATCATGCCAAGGCCAA | CCTGCAGGGAGGCCGAGCAT | 140 |
| TaPHOH | AF275551 | GGCCAGCAAAAAGCGCCTAGC | TGCCTGTCTGCTGCGCTCAT | 180 |
| TaDPE1 | DQ068045 | GGATGTCTCTTGGGCGCTAA | TGCTTCTTCGAGGCTAAGGC | 187 |
| TaDPE2 | BQ294920 | AGTATTCGGCGTGGTCTGTTCG | TGCTATGTCATCCAAGTCCCTA | 132 |
| TaRSR1 | JX473823 | GCCTGCAACTCCACCATG | CGGACGATGACGACGA | 284 |
| TaActin | AB181991 | AAACGAAGGATAGCATGAGGAAGC | AGCGGTCGAACAACTGGTA | 101 |
| TaGAPDH | EF592180 | TTTTCACCGACAAGGACA | AAGAGGAGCAAGGCAGTT | 179 |
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