**Table S1:** The primers used in the experiment

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene ID** | **Forward primer (5'-3')** | **Reverse primer (5'-3')** | **Role** |
| *HVA22* | CTCCACTGACGTAAGGGATGA | GTGATCTTTCTTGGGAGCGAC | Gene cloning |
| *qHVA22* | AGCAGCGGCAGCAAGGAG | GAGCGACGAAGGAGAGGAACC | qPCR |
| *Pinb-D1* | GGATTACGTGATGGAGCGATGT | CCGCTCTTCCACCATTTTGT |
| *18S* | TCGGGATCGGAGTAATGA | TTCGCAGTTGTTCGTCTT |
| *β-actin* | CCAAGGCGGAGTACGATGAGTCT | TTCATACAGCAGGCAAGCACCAT |

**Table S2:** The reaction mixture for quantitative real-time PCR

|  |  |
| --- | --- |
| **Components** | **Volume (μL)** |
| DNA template | 1.0 μL |
| Forward primer | 0.5 μL |
| Reverse primer | 0.5 μL |
| 2×RealStar Fast SYBR qPCR Mix | 10.0 μL |
| High/Low ROX Reference Mix | 0.4 μL |
| Sterile water | 7.6 μL |

**Table S3:** The primary endogenous genes in the recipient wheat showing high homology (> 95%) to the *TtHVA22* sequence

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **NO.** | **Description** | **Scientific Name** | **Max Score** | **Total Score** | **Query Cover** | **E value** | **Percent identity** | **Accession length** | **Accession** |
| 1 | PREDICTED: Triticum aestivum HVA22-like protein e (LOC123119536), mRNA | Triticum aestivum | 837 | 837 | 100% | 0 | 98.93 | 960 | [XM\_044539374.1](https://www.ncbi.nlm.nih.gov/nucleotide/XM_044539374.1?report=genbank&log$=nucltop&blast_rank=1&RID=KMWJ5PNF016) |
| 2 | Triticum aestivum TB2/DP1 protein (HVA22) mRNA, complete cds | Triticum aestivum | 837 | 837 | 100% | 0 | 98.93 | 471 | [KM030289.1](https://www.ncbi.nlm.nih.gov/nucleotide/KM030289.1?report=genbank&log$=nucltop&blast_rank=2&RID=KMWJ5PNF016) |
| 3 | Triticum aestivum mRNA, clone: whxi1c17, cultivar Chinese Spring | Triticum aestivum | 837 | 837 | 100% | 0 | 98.93 | 790 | [AK446595.1](https://www.ncbi.nlm.nih.gov/nucleotide/AK446595.1?report=genbank&log$=nucltop&blast_rank=3&RID=KMWJ5PNF016) |
| 4 | PREDICTED: Triticum aestivum HVA22-like protein e (LOC123110517), mRNA | Triticum aestivum | 773 | 773 | 100% | 0 | 96.79 | 952 | [XM\_044531056.1](https://www.ncbi.nlm.nih.gov/nucleotide/XM_044531056.1?report=genbank&log$=nucltop&blast_rank=4&RID=KMWJ5PNF016) |
| 5 | Triticum aestivum mRNA, clone: tplb0006h12, cultivar Chinese Spring | Triticum aestivum | 767 | 767 | 100% | 0 | 96.58 | 861 | [AK454668.1](https://www.ncbi.nlm.nih.gov/nucleotide/AK454668.1?report=genbank&log$=nucltop&blast_rank=5&RID=KMWJ5PNF016) |
| 6 | PREDICTED: Triticum aestivum HVA22-like protein e (LOC123106601), mRNA | Triticum aestivum | 737 | 737 | 100% | 0 | 95.51 | 902 | [XM\_044528707.1](https://www.ncbi.nlm.nih.gov/nucleotide/XM_044528707.1?report=genbank&log$=nucltop&blast_rank=6&RID=KMWJ5PNF016) |

This supplementary table lists only representative highly homologous entries (> 95% identity), confirming the existence of a multicopy conserved endogenous gene family that constitutes the primary background risk for off-target effects.

**Table S4:** *TtHVA22* Primer-BLAST results

|  |  |  |  |
| --- | --- | --- | --- |
| **Target gene** | **Primer sequence (5'->3')** | **Expected product** | **Specific validation** |
| *TtHVA22* | Forward primer: CTCCACTGACGTAAGGGATGA | 500 bp | NCBI Primer-BLAST showed no off-target matches in the RefSeq mRNA database restricted to *Triticum aestivum*. |
| Reverse primer: GTGATCTTTCTTGGGAGCGAC |

**Table S5:** The relative expression level (log2 fold change) of *Tel5E01G132800* in two wheat materials

|  |  |  |
| --- | --- | --- |
| **Wheat materials** | **Salt stress** | **Recovery** |
| “Y1805” | 7.33 ± 0.67a | 3.08 ± 0.23b |
| “Chinese Spring” | NE | NE |

“NE” indicates no expression. Values are the mean ± standard deviations of three replicates.

**Table S6:** *TtHVA22* copy number in the two overexpression lines

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ***TtHVA22* overexpression lines** | ***TtHVA22*** | | ***Pinb-D1*** | | **Ratio of log starting quantity** | **Copy number** |
| **Ct value** | **Log starting quantity** | **Ct value** | **Log starting quantity** |
| OE1 | 35.27 | 11.20 | 9.89 | 13.39 | 1.20 | 1 |
| OE2 | 36.25 | 10.85 | 9.94 | 13.37 | 1.23 | 1 |

**Table S7:** Correlations among wheat physiological and biochemical parameters under salt stress

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Index** | **Carotenoid** | **Chlorophyll b** | **Chlorophyll a** | **Relative conductivity** | **Malondialdehyde** | **Pyruvate** | **Peroxidase** | **Proline** | **Soluble proteins** | **Soluble sugars** |
| **Chlorophyll b** | 0.9297\*\*\* |  |  |  |  |  |  |  |  |  |
| **Chlorophyll a** | 0.9742\*\*\*\* | 0.9213\*\*\* |  |  |  |  |  |  |  |  |
| **Relative conductivity** | –0.8882\*\* | –0.7801\* | –0.9472\*\*\* |  |  |  |  |  |  |  |
| **Malondialdehyde** | –0.9535\*\*\*\* | –0.8651\*\* | –0.9834\*\*\*\* | 0.9760\*\*\*\* |  |  |  |  |  |  |
| **Pyruvate** | 0.9170\*\*\* | 0.9202\*\*\* | 0.9315\*\*\* | –0.8940\*\* | –0.9216\*\*\* |  |  |  |  |  |
| **Peroxidase** | 0.8411\*\* | 0.8012\*\* | 0.8740\*\* | –0.9209\*\*\* | –0.9063\*\*\* | 0.9639\*\*\*\* |  |  |  |  |
| **Proline** | 0.6674\* | 0.8247\*\* | 0.6247 | –0.4838 | –0.5775 | 0.8162\*\* | 0.7174\* |  |  |  |
| **Soluble proteins** | 0.9108\*\*\* | 0.9311\*\*\* | 0.9225\*\*\* | –0.8418\*\* | –0.8917\*\* | 0.9474\*\*\* | 0.8769\*\* | 0.7727\* |  |  |
| **Soluble sugars** | 0.8155\*\* | 0.7002\* | 0.8942\*\* | –0.9839\*\*\*\* | –0.9420\*\*\* | 0.8355\*\* | 0.8955\*\* | 0.4067 | 0.7915\* |  |
| **Abscisic acid** | 0.9829\*\*\*\* | 0.9458\*\*\* | 0.9786\*\*\*\* | –0.9145\*\*\* | –0.9672\*\*\*\* | 0.9584\*\*\*\* | 0.9033\*\*\* | 0.7329\* | 0.9348\*\*\* | 0.8601\*\* |

\**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001, and \*\*\*\**p* < 0.0001.

**Table S8:** Correlation between grain yield per plant and other traits in the *TtHVA22* overexpression lines in 2024 and 2025

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Agronomic traits** | **2024** | | **2025** | |
| **Correlation coefficient** | ***p*-value** | **Correlation coefficient** | ***p*-value** |
| Plant height | –0.7632\* | 0.0167 | –0.8561\*\* | 0.0032 |
| Stem diameter | 0.6739\* | 0.0466 | 0.7883\* | 0.0116 |
| Flag leaf length | 0.6851\* | 0.0417 | 0.9005\*\*\* | 0.0009 |
| Flag leaf width | 0.7976\* | 0.0100 | 0.8303\*\* | 0.0056 |
| Tiller number | 0.6724\* | 0.0472 | 0.8585\*\* | 0.0030 |
| Spike length | 0.7652\* | 0.0163 | 0.7422\* | 0.0220 |
| Spike number | 0.7263\* | 0.0267 | 0.8601\*\* | 0.0029 |
| 1,000-grain weight | 0.7887\* | 0.0115 | 0.8991\*\* | 0.0010 |

\**p* < 0.05, \*\**p* < 0.01, and \*\*\**p* < 0.001.