Mitogen-activated protein kinase TaMPK3 suppresses ABA response by destabilizing TaPYL4 receptor in wheat

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Article acceptance date: 10 June 2022

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Methods S1 Details of drought experiments

For drought treatments in the seedling stage, the size of the small red basin used was uniform, with a diameter of 12 cm, depth of 10.5 cm, and edge height of 2.5 cm. A total of 2,628 g of nutrient soil was fully mixed with 3 L of tap water. Then, 230 g of moist soil was put into each small red basin. The soil was pressed evenly until it was flush along the bottom edge of the basin. Each pot was dotted with 18 wheat (Triticum aestivum) seeds, with 1/6/11 seeds arranged in a circle and then covered to the top edge of the basin with equal amounts of soil. Wheat plants were grown in the greenhouse and no extra water was added during growth. Follow-up photos were taken at 6, 13, 15, and 16 days after planting. Soil water contents (%) [SWC% = 100*(soil fresh weight-Soil dry weight)/Soil dry weight] were measured at 6, 10, 12, 13, 15, 16, and 18 days after planting and at 3 days after rehydration (Table S2). Proline content was sampled at 12 days after planting and malondialdehyde (MDA) content was sampled at 15 days after planting. Rehydration started on the 18th day. Photos were taken after three days of rehydration and the survival rate and fresh weight of shoots were recorded. Drought treatment was performed with at least four replicates and physiological indexes were measured with three replicates of each line. Each experiment was repeated at least three times.

For drought treatment at the jointing stage in greenhouse conditions, the size of the white basin and dosages of soil and water used were uniform. The basin diameter was 21.5 cm, the depth was 17 cm, and the edge height was 4.5 cm. Each pot was dotted with six wheat seeds and provided 1 L of water every five days before drought treatments. As a drought stress treatment, water supply was withheld for 15 days at the jointing stage until the young leaves were wilt and old leaves were dry. The plants of each pot were then rewatered with 2 L of water every five days until fully grown and then photographed. Finally, agronomic traits of spikelet number, tiller number, panicle length, plant height, grain number per plant, grain weight per plant, thousand grain weight, grain width and grain length were determined. Three traits, grain number per plant, grain length, and grain width, were measured using an automatic seed counting and analyzing instrument (Model SC-G, Wanshen Ltd). Drought treatment was performed with at least three replicates and agronomic traits were measured for 15 plants per line. Statistical analysis using one-way ANOVA with multiple comparisons
revealed the significant differences compared with WT (*p < 0.05, **p < 0.01).

For drought treatment in the field, the seeds of TaMPK3-RNAi (i-1 and i-3), WT, and TaMPK3-overexpressing (OE-2 and OE-11) lines were planted at the experimental station (40°13’52”N, 116°33’52”E) of the Institute of Crop Sciences, CAAS, Beijing. Rainfall data in the region is listed in Tables S3 and 4. Soil moisture content data in the field was calculated (Table S5). For the field experiment, TaMPK3 transgenic plants and WT were sown at the end of February and then mulched until mid-March and harvested the following July. In the watered treatment, an additional instance of irrigation was applied during the jointing and filling stages. In the drought treatment, no additional irrigation was applied from sowing to harvest. In order to prevent the impact of rainfall during the drought treatment after flowering, we expanded the area in advance to keep out rain according to the weather forecast. Other crop management was consistent with local cultivation practices for wheat varieties in the field. The major agronomic traits of plant height, the tiller number, the effective tiller number, the grain number per plant, the panicle length, the spikelet number, the grain length, the grain width, and the thousand grain weight of TaMPK3-RNAi, WT, and TaMPK3-overexpressing wheat lines under drought stress and well-watered condition in the field were collected. Statistical analysis using one-way ANOVA with multiple comparisons revealed the significant differences compared with WT (*p < 0.05, **p < 0.01). The agronomic traits of the transgenic wheat and WT were investigated for 10 plants per line in each experiment.
Fig. S1 Alignment and domain analysis of MPK3. Amino acid sequences from multiple genomic copies, along with the accessions reported in Table S7, of identified MPKs were aligned in DNAMAN under default settings. The PK domain was marked with a line segment below the alignment sequences and the ‘MAP kinase’ signature (specific to MPKs), contained in the PK domain, was marked with an orange rectangle. Straight lines were used to exhibit the positions of different feature sequences. Highlighted sequences with red straight lines were the ATP binding signature, the catalytic C-loop, the activation T-loop, CD domain, and EF-hand CBP. Sequence deviations from the conserved MPK motifs of plant MPKs were marked in red font in feature sequences.
Fig. S2 Molecular phylogenetic analysis of plant MPK3 genes. TraesCS4D02G198600 was used as a direct query in Ensembl (http://plants.ensembl.org/index.html) to search for homologous genes in typical monocots (pink) and dicots (blue). The full-length amino acid sequences of MPK3 of each species were aligned in MEGA5 by Clustal W under default settings, using MPK3 homologue of *Sphagnum fallax* (Sphfalx0169s0005, the gene ID in Phytozome) as an outgroup. The resulting alignments in meg format were submitted to MEGA5 to generate a Maximum Likelihood bootstrapped tree based on the Jones-Taylor-Thornton (JTT) matrix-based model. A discrete Gamma distribution was used
to model evolutionary rate differences among sites. To identify the species of origin for each MPK3, the name of the protein was replaced with the corresponding species name. The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) were shown next to the branches. Related sequence information is listed in Table S7.
Fig. S3 Amino acid sequence alignment of TaPYLs. The accessions of these amino acid sequences except TaPYL9 (A0A3B6RID7) are listed in Table S1. Identified TaPYLs were aligned in DNAMAN under default settings.
Fig. S4 The subcellular location of nine TaPYLs. Localization of TaPYL proteins under normal conditions. Images were observed under a confocal laser scanning microscope (LSM700, Zeiss). Scale bars = 10 μm.
Fig. S5 MPK3-PYL module exists widely in monocots and dicots. (a) BiFC assays in tobacco (*Nicotiana benthamiana*) showing that TaMPK3-nYFP interacted with TaPYL4-cYFP, AtMPK3-nYFP interacted with AtPYL4-cYFP, GmMPK3-nYFP interacted with GmPYL4-cYFP, and OsMPK3-nYFP interacted with OsPYL9-cYFP. Scale bars = 50 μm. (b) LCI assays demonstrating that AtMPK3-nLUC interacted with AtPYL4-cLUC, GmMPK3-nLUC interacted with GmPYL4-cLUC, and OsMPK3-nLUC interacted with OsPYL4-cLUC in tobacco.
Fig. S6 Overexpression and RNAi of TaMPK3 in wheat (Triticum aestivum). (a) Relative expression level of 16 TaMPK3-overexpressing lines using qPCR assays. Three independent TaMPK3-overexpressing lines (OE-2, OE-6, and OE-11) with the highest expression level were selected for functional study. The expression of β-actin was analyzed as an internal control. Each data point was the mean (±SD) of three experiments. (b) Relative expression level of 15 TaMPK3-RNAi lines using qPCR assays. Statistical analysis using one-way ANOVA with multiple comparisons revealed the significant differences compared with WT (*p < 0.05, **p < 0.01). Three independent TaMPK3-RNAi lines (i-1, i-3, and i-10) with the lowest expression level were selected for research. The expression of β-actin was analyzed as an internal control. Each data point was the mean (±SD) of three experiments. (c-d) Sketches of T-DNA insertion sites of the selected transgenic lines in the wheat genome detected by mhiTAIL-PCR. The mhiTAIL-PCR products purified from agarose gels were cloned into pEASY®-Blunt Zero Cloning vector (TransGen, Catalog no. CB501-01), then six monoclonal colonies were selected for sequencing with universal primers (M13F and M13R). The T-DNA insertions are shown as colored triangles and the flanking genomic sequences are in lower-case letters. The numbers at the bottom indicate T-DNA insertion sites (bp) and the positions of the wheat genome.
Fig. S7 Wheat (*Triticum aestivum*) plants overexpressing *TaMPK3* showed reduced ABA sensitivity. (a-c) Phenotypes of *TaMPK3*-overexpressing (OE-2, OE-6, and OE-11) and WT wheat plants under control and different concentrations of ABA treatments after soaking. Photographs were taken after 2 days of incubation in the greenhouse. (d) Bar graphs of seedling shoot length under control and ABA treatments were analyzed. Each treatment had at least three independent replicates and each replicate contained eight plants. Each data point is the mean (±SD) of 15 seedlings. Statistical analysis using one-way ANOVA with multiple comparisons revealed the significant differences compared with WT (*p* < 0.05, **p** < 0.01).
Fig. S8 The impact of ABA treatment on the interaction between TaMPK3 or TaMPK3\textsuperscript{K65R} and TaPYL4. (a-b) LCI assays demonstrating that TaPYL4-cLUC directly interacted with TaMPK3-nLUC (a) and TaMPK3\textsuperscript{K65R}-nLUC (b) in tobacco (\textit{Nicotiana benthamiana}) and ABA diminished the interaction. YB2-nLUC and YA16-cLUC was used as positive control (c). The dotted circles represent the injection areas. Three biological replications were performed with similar results. Each data point was the mean relative luciferase activity (±SD) of three leaves. Statistical analysis using Student’s \textit{t}-test revealed the significant differences (**p < 0.01) between control and 10 μM ABA treatments.
The S/TP site analysis of TaPYLs. (a) The S/TP site in TaPYLs was marked with a purple square. The accessions of these amino acid sequences except TaPYL9 (A0A3B6RID7) are listed in Table S1. (b) Three TaPYL4 identified from A, B, and D sub-genomes were aligned in Geneious under default settings. The S/TP site in TaPYL4 was marked with a purple square. The mutated serine site of TaPYL4 was marked with an orange square.
**Fig. S10** The interaction between different mutant combination of TaMPK3 and TaPYL4. LCI assays demonstrating that TaMPK3<sup>K65R</sup>-nLUC interacted with TaPYL4-cLUC, TaMPK3<sup>D191G/E195A</sup>-nLUC interacted with TaPYL4-cLUC, TaMPK3-nLUC interacted with TaPYL4<sup>M1</sup>-cLUC, and TaMPK3-nLUC interacted with TaPYL4<sup>M2</sup>-cLUC in tobacco (*Nicotiana benthamiana*). TaPYL4<sup>M1</sup> stands for TaPYL4<sup>S58A</sup>, TaPYL4<sup>M2</sup> stands for TaPYL4<sup>T3A/T177A</sup>. 

| TaMPK3<sup>K65R</sup> nLUC + TaPYL4 cLUC | TaMPK3<sup>D191G/E195A</sup> nLUC + TaPYL4 cLUC | TaMPK3 nLUC + TaPYL4<sup>M1</sup> cLUC | TaMPK3 nLUC + TaPYL4<sup>M2</sup> cLUC | TaMPK3 nLUC + TaPYL4<sup>S58A</sup> cLUC | TaMPK3 nLUC + TaPYL4<sup>T3A/T177A</sup> cLUC | 
|-------------------------------------|-----------------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
Fig. S11 ABA and PEG induced the accumulation of TaMPK3 at both transcriptional and protein levels. (a-b) The expression profile of TaMPK3 in different wheat (*Triticum aestivum*) tissues (a) and under control, ABA, and PEG6000 treatments for the indicated growth stages (b). Transcript levels were quantified by qPCR assays. The expression of β-actin was analyzed as internal control. Each data point is the mean (±SD) of three replicates. Related primers are listed in Table S1. (c-e) Protein level of TaMPK3 in 7-day-old wheat seedlings after control (c), ABA (d), and PEG6000 (e) treatments for the indicated times. Total proteins were extracted and subjected to immunoblot analysis with p44/42 MAPK (Erk1/2) antibody (CST, Catalog no. 4695S). Rubisco was used as a loading control.
Fig. S12 The specificity of anti-Erk1/2 on TaMPK3 protein.

TaMPK3-OE, TaMPK3-RNAi, and WT was the total protein of TaMPK3-overexpressing, RNAi and Fielder wheat (*Triticum aestivum*) plants, respectively. The purified TaMPK3-MBP protein was used as positive control. Rubisco was used as a loading control.
Fig. S13 The responses of TaMPK3 and TaPYL4 during drought and/or post-drought recovery stage. (a-b) The phenotypes of wheat (*Triticum aestivum*) suffering different degrees of drought stress. Ten-day-old wild-type (WT) wheat seedlings were provided different degrees of drought stress: control (CK) with ~225% of soil water content; drought condition I (D1) with ~68% of the soil water content; drought condition II (D2) with ~47% of the soil water content; drought condition III (D3) with ~31% of soil water content; and subsequently rehydrated (RH) for 0.5 h, 1 h, 2 h, and 4 h with ~400% of the soil water content. Total RNA and protein were extracted from each sample. Each data point was the mean (±SD) of six biological replicates. Statistical analysis using Student’s *t*-test revealed the significant differences of soil water content (**)p < 0.01) between CK and different drought conditions. (c-e) The relative expression level of TaMPK3 under different degrees of
drought stress (c), subsequently rehydrated for the indicated times after D2 (d), and subsequently rehydrated for the indicated times after D3 (e). Each data point was the mean (±SD) of three biological replicates. Statistical analysis using Student’s t-test revealed the significant differences of TaMPK3 expression (*p < 0.05, **p < 0.01) between CK and different sampling points. (f) In vitro cell-free protein degradation assays, showing degradation of 10 μg TaPYL4-GST after 0.5 h in 50 μg protein extracts from WT wheat plants under CK and rehydrated for 0.5 h, 1 h, 2 h, and 4 h after D3. Immunoblots were probed with anti-GST antibody. Rubisco was used as a loading control. Input indicated 50% of TaPYL4 in degradation reaction. (g) The protein level of TaMPK3 in 50 μg protein extracts from WT wheat plants under CK and rehydrated for 0.5 h, 1 h, 2 h, and 4 h after D3. Immunoblots were probed with anti-Erk1/2 antibody.
Fig. S14 The agronomic traits of TaMPK3-RNAi, WT, and TaMPK3-overexpressing wheat (Triticum aestivum) in greenhouse under watered condition. (a) The phenotypic analysis of TaMPK3-RNAi (i-1, i-3, and i-10), WT, and TaMPK3-overexpressing (OE-2, OE-6, and OE-11) wheat plants under watered condition in greenhouse. (b-j) The spikelet number (b); the tiller number (c); the panicle length (d); the plant height (e); the grain number per plant (f); the grain weight per plant (g); the thousand grain weight (h); the grain width (i); and the grain length (j) of TaMPK3-RNAi, WT, and TaMPK3-overexpressing wheat lines under watered condition in greenhouse. Each treatment had four independent replicates and each replicate contained six plants. Each data point was the mean (±SD) of 10 independent samples. Statistical analysis using one-way ANOVA with multiple comparisons revealed the significant differences compared with WT (**p < 0.01).
Fig. S15 The agronomic traits of *TaMPK3*-RNAi, WT, and *TaMPK3*-overexpressing wheat (*Triticum aestivum*) in the field under watered condition. (a-b) The phenotypes (a) and the seeds (b) of *TaMPK3*-RNAi (i-1 and i-3), WT, and *TaMPK3*-overexpressing (OE-2 and OE-11) wheat plants under watered conditions. (c-j) Plant height (c); grain length (d); grain width (e); thousand grain weight (f); effective tiller number (g); spikelet number (h); panicle length (i); and spikelet density (j) of *TaMPK3*-RNAi, WT, and *TaMPK3*-overexpressing wheat lines under watered conditions. Each treatment had three independent replicates and each replicate contained 15 plants. Each data point was the mean (±SD) of 10 independent samples. Statistical analysis using one-way ANOVA with multiple comparisons revealed the significant differences compared with WT (**p < 0.01).
Fig. S16 TaMPK3 interacted with TaCaM. (a) The interactions between TaMPK3-nYFP and TaCaM-cYFP using BiFC assays in wheat (*Triticum aestivum*) mesophyll protoplasts. Scale bars = 10 μm. (b) LCI assays demonstrating that TaMPK3-nLUC directly interacted with TaCaM-cLUC in tobacco (*Nicotiana benthamiana*).
| Primer name | Primer sequence | Application | UniProtKB ID |
|-------------|----------------|-------------|--------------|
| TaPYL1-F    | TGCTCCAAGCCTCTCTCTCG | PCR         | A0A3B5YX52   |
| TaPYL1-R    | CAGCACCTCAGAATCACACC | PCR         |              |
| TaPYL2-F    | AGAGGAAGACATGGAGGCC | PCR         |              |
| TaPYL2-R    | GAGAAACCAGAAGCATGAACTCAC | PCR | A0A3B6EHC1 |
| TaPYL3-F    | CCAACCCCCATCAAGGAC | PCR         |              |
| TaPYL3-R    | GGGCGAAGAAAAACACAGAA | PCR        | A0A3B6TMH9   |
| TaPYL4-F    | TTACTCCCAAACCCACCCA | PCR         |              |
| TaPYL4-R    | GCCGAAAAGAAACAGAAGGTA | PCR        | A0A3B6D9V8   |
| TaPYL5-F    | CTTCTTCACAGCGAAAATCAG | PCR        |              |
| TaPYL5-R    | AGATTCCAGAACCACACCTTT | PCR       | A0A077RWR8   |
| TaPYL6-F    | GTCCATCTACCCAGTACAGC | PCR         |              |
| TaPYL6-R    | CACAAGGAGTCAGGAAACAAATAAG | PCR | A0A3B5ZX84 |
| TaPYL7-F    | ACACGGCAGAAGAAAAAGC | PCR         |              |
| TaPYL7-R    | GAGACCCAATGGGAGGAAA | PCR         | A0A3B6HSL7   |
| TaPYL8-F    | CGATTAGCCCAACCCAGC  | PCR         |              |
| TaPYL8-R    | ACAAGTCCCTGCCCGTGA | PCR         | A0A3B5ZSS4   |
| TaMPK3-F    | CTCACACTCCTCACCGTGGC | PCR        |              |
| TaMPK3-R    | GAAATCATACATTGAGGGTAAC TA | PCR | A0A3B6JLL7 |
| AtPYL4-F    | GAAAGGCCAGCAGCACCACACT | PCR | O80920       |
| AtPYL4-R    | CAACGCACAAAGACTCATCAG | PCR         |              |
| AtMPK3-F    | CTCACAGTATCTACTCTCAGACCT | PCR | Q39023       |
| AtMPK3-R    | AGACAAACTCAGCGACGAG | PCR         |              |
| GmMPK3-F    | CCTCTTCCTCCACTTCTCC | PCR         |              |
| GmMPK3-R    | CTTGTACGTGTTCTCGTGGT | PCR         |              |
| GmPYL4-F    | TGCCCACCTCTTGCAA | PCR         |              |
| GmPYL4-R    | AGAACAGAACCCATACC | PCR         |              |
| OsMPK3-F    | CTCTAGCTTTGCTGGTCTCTC | PCR | K7MTN5       |
| OsMPK3-R    | AGTTCAACACCTCCTTATTCG | PCR | Q10N20       |
| OsPYL9-F    | CGCACCACAAAGCAAAG | PCR         |              |
| OsPYL9-R    | GGCCTCAAAGGAGGACACA | PCR         | Q6EN42       |
| mLAD1       | GCTACAGATGGACTGCTGAGTGCACCTG(G/C/A) N (G/C/A) NNGGAA | Tail-PCR | |
| mLAD2       | GCTCAGATGGACTGCTGAGTGCACCTG(G/C/T) N (G/C/T) NNCCTT | Tail-PCR | |
| mLAD3       | GCTCAGATGGACTGCTGAGTGCACCTG(G/T/A) N (G/T/A) NNCACC | Tail-PCR | |
| mLAD4       | GCTCAGATGGACTGCTGAGTGCACCTG(G/T/A) N (G/T/A) NNTTGG | Tail-PCR | |
| mAC0        | GAGCTCAGATGGACTGCTGAGTGCACCTG(G/C/T) N (G/C/T) NNCCTT | Tail-PCR | |
| mAC1        | CGATGGACTGCTGAGTGCACCTG(G/C/T) N (G/C/T) NNCCTT | Tail-PCR | |
| RB-0a       | TAAATGCGCTTTGACACATCCTCCCTC (160 bp from RB) | Tail-PCR | |
| RB-1a       | CGATGGACTGCTGAGTGCACCTGCTGTAATAGCAGGAAGGACGC | Tail-PCR | |
| RB-2a       | AGTGGCGCAGCTGAAATGGCGAATG (80 bp from RB) | Tail-PCR | |
| Primer Name | Sequence | Source |
|-------------|----------|--------|
| LB-0a       | ATGACGTGGTTTCTGGCAGCTGGACTT (334 bp from LB) | Tail-PCR |
| LB-1a       | CGATGGACTGCTGAGTGGCACCTGGTCTGCTGCCGTCACCAGAGTTT (281 bp from LB) | Tail-PCR |
| LB-2a       | TCCAGTACTAAAATCCAGATCCCCGAA (97 bp from LB) | Tail-PCR |
| RT-TaPP2C1-F | TGTGCCGCGATTCTTCAGCTTG | qPCR |
| RT-TaPP2C1-R | CCCCCATCTGCTGAATCTCCTCACC | qPCR |
| RT-TaPP2C2-F | GGAAGATGAGCGGTGCCAGGATTGA | qPCR |
| RT-TaPP2C2-R | CAACCTGGCTACGTCCTTGATTTGACA | qPCR |
| RT-TaPP2C6-F | ACGAGTGCTGATCCCGAGCCAG | qPCR |
| RT-TaPP2C6-R | GGAGATGGTTGCTCGAAGGTCTTTT | qPCR |
| RT-TaDHN3-F | CATTTCGAGCCACCGAGA | qPCR |
| RT-TaDHN3-R | GGGCCACCACGGAGGTTT | qPCR |
| RT-TaPOD21-F | CGGTGCTGACGCTGAACCTT | qPCR |
| RT-TaPOD21-R | CCACGTACTGAGTTGCGCA | qPCR |
| RT-TaActin-F | CCTCTCTGCGCCAATCGT | qPCR |
| RT-TaActin-R | TCACCGAGCGGAATCTT | qPCR |
| RT-TaMPK3-F | AGATGGTGCAATCGAGAGA | qPCR |
| RT-TaMPK3-R | GCCTACTATGTTTCTCGTGTCG | qPCR |
Table S2 Soil water content (%) during the drought treatments at the seedling stage

|       | i-1          | i-3          | i-10         | WT           | OE-2         | OE-6          | OE-11         |
|-------|--------------|--------------|--------------|--------------|--------------|---------------|---------------|
| 6 d   | 206.34±0.85  | 201.94±4.08  | 206.51±2.14  | 205.51±4.64  | 203.45±1.48  | 203.95±0.59   | 206.94±1.01   |
| 10 d  | 146.76±2.60  | 145.55±3.57  | 143.44±1.74  | 153.49±7.59  | 141.33±6.43* | 141.01±9.55*  | 150.40±9.96   |
| 12 d  | 76.22±0.39   | 75.23±1.02   | 75.11±1.45   | 76.34±0.61   | 67.97±0.79** | 70.18±1.37**  | 68.10±0.59**  |
| 13 d  | 59.28±0.59   | 60.59±0.41** | 59.04±0.80   | 59.48±0.51   | 52.65±0.54***| 51.10±0.66**  | 51.67±0.35**  |
| 15 d  | 50.49±0.60   | 51.80±0.39** | 50.30±0.63   | 50.61±0.44   | 43.81±0.58** | 42.33±0.71**  | 42.85±0.40**  |
| 16 d  | 41.52±0.43   | 42.91±0.36** | 41.52±0.52   | 41.77±0.42   | 35.11±0.44** | 33.66±0.50**  | 33.91±0.28**  |
| 18 d  | 29.12±0.47   | 30.20±0.32** | 28.90±0.61   | 29.14±0.34   | 23.23±0.41** | 21.96±0.49**  | 22.32±0.39**  |
| 3 d   | 381.02±1.30  | 375.81±3.01  | 380.94±3.28  | 379.23±6.91  | 376.41±2.32  | 377.15±0.83  | 381.35±0.99   |

* Each data was the mean (±SD) of six independent samples. Statistical analysis using one-way ANOVA with multiple comparisons revealed the significant differences compared with WT (*p < 0.05, **p < 0.01).

Table S3 Annual rainfall data from 2010-2020 at the experimental station (40°13’52’’N, 116°33’52’’E) of the Institute of Crop Sciences, CAAS, Beijing

| Year | Rainfall (mm) | Station name                                      | Station number |
|------|---------------|--------------------------------------------------|----------------|
| 2010 | 666.75        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2011 | 788.67        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2012 | 773.43        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2013 | 661.92        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2014 | 524.51        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2015 | 483.36        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2016 | 735.08        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2017 | 710.44        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2018 | 672.34        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2019 | 490.47        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
| 2020 | 528.57        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH        | 54511099999    |
Table S4 Monthly rainfall data from 2018-2020 at the experimental station (40°13’52”N, 116°33’52”E) of the Institute of Crop Sciences, CAAS, Beijing

| Year | Month | Rainfall (mm) | Station name | Station number |
|------|-------|---------------|--------------|----------------|
| 2020 | 01    | 5.08          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 02    | 37.59         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 03    | 12.45         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 04    | 12.7          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 05    | 49.53         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 06    | 33.78         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 07    | 109.22        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 08    | 167.64        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 09    | 72.64         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 10    | 0.0           | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 11    | 27.94         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2020 | 12    | 0.0           | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 01    | 0.0           | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 02    | 2.29          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 03    | 2.54          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 04    | 43.18         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 05    | 84.84         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 06    | 12.19         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 07    | 105.16        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 08    | 70.36         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 09    | 116.08        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 10    | 26.16         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 11    | 21.84         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2019 | 12    | 5.84          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 01    | 0.0           | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 02    | 0.0           | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 03    | 7.87          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 04    | 54.86         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 05    | 12.7          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 06    | 45.21         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 07    | 364.74        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 08    | 136.65        | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 09    | 40.64         | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 10    | 8.38          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 11    | 0.76          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
| 2018 | 12    | 0.51          | BEIJING CAPITAL INTERNATIONAL AIRPORT, CH | 54511099999 |
### Table S5 Soil water content (%) in the field

| Soil depth             | 10-20 cm | 20-40 cm |
|------------------------|----------|----------|
| Well-watered condition | 27.16±1.41 | 35.63±2.13 |
| Drought condition      | 10.23±0.91 | 13.96±1.08 |

Each data was the mean (±SD) of six independent samples.

### Table S6 The list of the interacting candidates of TaPYL4

| Gene ID                  | Homologs      | Name     | Species     | Description                                                                 | Identity     |
|--------------------------|---------------|----------|-------------|-----------------------------------------------------------------------------|--------------|
| TraesCS1A02G151000       | LOC_Os10g33270| OsCYN    | Oryza sativa| Similar to Cyanate lyase (CYN)                                              | 85.35        |
| TraesCS1A02G409900       | LOC_Os01g46950| null     | Oryza sativa| Glycoside hydrolase-type carbohydrate-binding, subgroup domain containing protein | 79.167       |
| TraesCS1D02G150300       | -             | -        | -           | Death-associated protein kinase 1                                           | -            |
| TraesCS2A02G090000       | LOC_Os07g49150| TBPOs-2  | Oryza sativa| Similar to 26S proteasome subunit 4-like protein (26S proteasome subunit AtRPT2a) | 97.773       |
| TraesCS2A02G212200       | LOC_Os02g02820| TDR      | Oryza sativa| Basic helix-loop-helix (bHLH) transcription factor, Tapetum development and degeneration | 27.734       |
| TraesCS2A02G499400       | LOC_Os04g56480| LML1     | Oryza sativa| Similar to OSIGBa0132E09-OSIGBa0108L24.21 protein                          | 89.683       |
| TraesCS2A02G523300       | LOC_Os04g54810| null     | Oryza sativa| Similar to OSIGBa0147H17.8 protein                                           | 87.649       |
| TraesCS2B02G392900       | LOC_Os04g44440| OsTCP17  | Oryza sativa| Similar to Auxin-induced basic helix-loop-helix transcription factor         | 75.463       |
| TraesCS2B02G416900       | LOC_Os04g48416| OsSub45  | Oryza sativa| Similar to OSIGBa0147H17.8 protein                                           | 87.143       |
| TraesCS2D02G112900       | LOC_Os07g47620| OsUsp1   | Oryza sativa| Rossmann-like alpha/beta/alpha sandwich fold domain containing protein       | 68.519       |
| TraesCS2D02G417000       | LOC_Os04g52540| OsAGO2   | Oryza sativa| Argonaute and Dicer protein, PAZ domain containing protein                   | 74.803       |
| TraesCS3B02G297300       | LOC_Os01g49290| RACK1    | Oryza sativa| Protein containing the WD-40 repeat, Innate immunun                           | 90.991       |
| TraesCS3D02G264000       | LOC_Os01g49290| RACK1    | Oryza sativa| Protein containing the WD-40 repeat, Innate immunun                           | 90.991       |
| TraesCS3D02G417500       | LOC_Os01g68770| -        | Oryza sativa| Similar to Selenium binding protein                                          | 93.79        |
| TraesCS4A02G326100       | LOC_Os03g63520| null     | Oryza sativa| Der1-like domain containing protein                                          | 95.618       |
| TraesCS4B02G143200       | LOC_Os11g47670| null     | Oryza sativa| Thaumatin, pathogenesis-related family protein                               | 68.367       |
| TraesCS4D02G107400       | LOC_Os11g07020| Aldo     | Oryza       | Fructose-bisphosphate aldolase, chloroplast                                   | 93.041       |
| Accession       | Gene ID          | Description                                                                 |
|-----------------|------------------|------------------------------------------------------------------------------|
| TraesCS4D02G198600 | AT3G45640        | MPK3, Arabidopsis, Mitogen-activated protein kinase 3                         |
| TraesCS5A02G319800 | LOC_Os09g36190   | Oryza sativa, Similar to Glycosyltransferase QUASIMODO1 (EC 2.4.1.-)         |
| TraesCS5B02G012100 | LOC_Os03g14450   | Oryza sativa, Similar to Enolase 2 (EC 4.2.1.11)                             |
| TraesCS5B02G509500 | AT1G56070        | LOS1, Arabidopsis, Ribosomal protein S5/Elongation factor G/III/V family protein |
| TraesCS6A02G213700 | LOC_Os04g40950   | Oryza sativa, Similar to Glyceraldehyde-3-phosphate dehydrogenase, cytosolic 3 (EC 1.2.1.12) |
| TraesCS6D02G196300 | LOC_Os04g40950   | Oryza sativa, Similar to Glyceraldehyde-3-phosphate dehydrogenase, cytosolic 3 (EC 1.2.1.12) |
| TraesCS7A02G164000 | LOC_Os06g10230   | Oryza sativa, Receptor-like kinase, Heat tolerance                           |
| TraesCS7A02G338500 | LOC_Os05g25770   | Oryza sativa, WRKY transcription factor, Benzothiadiazole (BTH)-inducible blast resistance |
| TraesCS7B02G193700 | LOC_Os06g22070   | Oryza sativa, Mitochondrial glycoprotein family protein                     |
| TraesCS7B02G208700 | LOC_Os08g04180   | Oryza sativa, Similar to Tryptophan synthase beta chain 1 (EC 4.2.1.20)      |
| TraesCS7D02G054600 | LOC_Os06g03780   | Oryza sativa, WD40 repeat-like domain containing protein                    |
| TraesCS7D02G264000 | LOC_Os09g33520   | Oryza sativa, Conserved hypothetical protein                                |
| Species                    | Target % identity | Gene Name | Gene ID_Ensembl                  | Transcript ID_Ensembl | Location                  |
|---------------------------|-------------------|-----------|----------------------------------|-----------------------|--------------------------|
| Aegilops tauschii         | 77.47%            | AetMPK3   | AET4Gv20512600                  | AET4Gv20512600.2     | 4D:352,872,512-352,875,62 |
| Arabidopsis thaliana      | 72.16%            | ATMKP3    | AT3G45640                        | AT3G45640.1           | 3:16,756,571-16,758,874:1 |
| Beta vulgaris             | 69.55%            | -         | BVRB_9g207340                    | KMT02155             | 9:13,111,614-13,116,118:-1 |
| Brachypodium distachyon   | 98.37%            | BdMPK3    | BRADI_1g65810v3                   | KQK22200             | 1:64,962,458-64,965,083:1 |
| Brassica oleracea         | 72.43%            | -         | Bo3g130040                       | Bo3g130040.1         | C3:47,472,433-47,473,965:1 |
| Brassica rapa             | 74.77%            | -         | Bra038281                         | Bra038281.1-P        | A06:11,393,911-11,395,465 |
| Cucumis sativus           | 74.86%            | -         | Csa_1G479630                      | KGN65657             | 1:17,309,108-17,313,066:1 |
| Glycine max               | 74.93%            | GmMPK3    | GLYMA_12G073000                  | KRH24954             | 12:5,388,370-5,391,780:-1 |
| Hordeum vulgare           | 99.19%            | HvMPK3    | HORVU4Hr1G057200                 | HORVU4Hr1G057200.4   | chr4H:480,892,997-480,895 |
| Oryza sativa Indica Group | 91.60%            | OsMPK3    | BGIOSGA010959                     | BGIOSGA010959-TA     | 3:10,741,695-10,743,976:-1 |
| Oryza sativa Japonica Group | 91.33%         | Os03g0285800 | Os03t0285800-01 | Os03t0285800-01 | 3:9,847,723-9,850,384:-1 |
| Phaseolus vulgaris        | 74.12%            | -         | PHAVU_011G071400                  | ESW04159             | 11:6,384,955-6,388,514:1  |
| Setaria italica           | 90.67%            | SiMPK3    | SETIT_036218mg                    | KQK91364             | IX:49,447,133-49,452,474:1 |
| Solanum lycopersicum      | 71.58%            | -         | Solvc06g005170.3                  | Solvc06g005170.3     | 6:191,876-195,121:1       |
| Solanum tuberosum         | 71.58%            | StMPK3    | PGSC0003DMG4000                   | PGSC0003DMT4000      | 6:7,463,480-7,467,923:1   |
| Sorghum bicolor           | 91.18%            | SbMPK3    | SORB1_3001G410100                 | KXG39626             | 1:69,359,089-69,363,908:1 |
| Triticum dicoccoides      | 83.33%            | -         | TRIDC4AG014520                    | TRIDC4AG014520.1     | 4A:120,373,811-120,376,46 |
| Triticum dicoccoides      | 99.73%            | -         | TRIDC4BG036510                    | TRIDC4BG036510.1     | 4B:433,198,487-433,201,52 |
| Triticum turidum          | 96.04%            | -         | TRITD4Av1G049530                  | TRITD4Av1G049530.4   | 4A:118,767,870-118,770,32 |
| Triticum turidum          | 92.23%            | -         | TRITD4Bv1G124550                  | TRITD4Bv1G124550.2   | 4B:436,012,842-436,015,17 |
| Triticum urartu           | 86.82%            | TuMPK3    | TRIUR3_20327                      | TRIUR3_20327-T1      | scaffold11819:175,440-178,036:-1 |
| Zea mays                  | 90.11%            | ZmMPK3    | Zm00001d047758                    | Zm00001d047758_T0    | 9:141,026,557-141,029,745:1 |
| Triticum aestivum         | 99.73%            | 4A        | TraesCS4A02G10640                 | TraesCS4A02G10640.0  | 4A:120604688-120608133:-1 |
| Triticum aestivum         | 99.73%            | 4B        | TraesCS4B02G19780                 | TraesCS4B02G19780.0  | 4B:426767061-426769719:1  |
| Triticum aestivum         | 99.73%            | 4A        | TraesCS4A02G10640                 | TraesCS4A02G10640.0  | 4A:120604688-120608133:-1 |
| Triticum aestivum         | 99.73%            | 4B        | TraesCS4B02G19780                 | TraesCS4B02G19780.0  | 4B:426767061-426769719:1  |
**Table S8 The promoter of TaMPK3 gene**

| TaMPK3_4A_promoter | TaMPK3_4B_promoter | TaMPK3_4D_promoter | Function | Sequence |
|--------------------|--------------------|--------------------|----------|----------|
| A-box              | A-box              | A-box              | cis-acting regulatory element | CCGTCC   |
| ABRE               | -                  | ABRE              | cis-acting element involved in the abscisic acid responsiveness | ACGTG or GCCGCGTGGC |
| -                  | -                  | AC-I              | -        | (T/C)(T/C)(T/C)ACC(T/C)A CC |
| ARE                | ARE                | ARE               | cis-acting regulatory element essential for the anaerobic induction | AAACCA   |
| -                  | as-1              | -                 | -        | TGACG    |
| AT-TATA-box        | AT-TATA-box       | AT-TATA-box       | part of a conserved DNA module involved in light responsiveness | ATTAAT   |
| Box 4              | -                  | -                 | common cis-acting element in promoter and enhancer regions | CAAAT or TGCCAA or CCAAT |
| CAAT-box           | CAAT-box          | CAAT-box          | cis-acting regulatory element related to meristem expression | GCCACT   |
| CAT-box            | -                  | -                 | -        | CCAACGG  |
| CCAAT-box          | CCAAT-box         | CCAAT-box         | MYBHv1 binding site | CCGTCC   |
| CCGTCC-box         | CCGTCC-box        | CCGTCC-box        | -        | CCGTCC   |
| circadian          | -                  | circadian         | cis-acting regulatory element involved in circadian control | CAAAGATATC |
| -                  | CGTCA-motif       | CGTCA-motif       | cis-acting regulatory element involved in the MeJA-responsiveness | CGTCA    |
| DRE core           | DRE core          | DRE core          | -        | GCCGAC   |
| G-Box              | -                  | -                 | cis-acting regulatory element involved in light responsiveness | CACGTT   |
| G-box              | G-box             | G-box             | cis-acting regulatory element involved in light responsiveness | CACGAC or CACGTC |
| GC-motif           | -                  | -                 | enhancer-like element involved in anoxic specific inducibility | CCCCCC   |
| MBS                | -                  | -                 | MYB binding site involved in drought-inducibility | CAACTG   |
| -                  | GT1-motif         | GT1-motif         | light responsive element | GGTAAA   |
| -                  | JERE              | -                 | -        | AGACCGCC |
| MYB                | MYB               | MYB               | -        | CAACCA   |
| MYB                | MYB               | MYB               | -        | CCGTTG   |
| recognition site   | recognition site  | recognition site  | -        | CAACACAG |
| Myb-binding site   | Myb-binding site  | Myb-binding site  | -        | TAACTG or CACTG |
| Myb                | -                  | -                 | -        | TAACTG   |
| -                  | MYB-like          | MYB-like          | -        | TAACCA   |
| MYC | MYC | MYC | CATTTG |
| Sp1 | Sp1 | Sp1 | light responsive element | GGGCGG |
| -   | P-box | -   | gibberellin-responsive element | CTTTTG |
| STRE | STRE | STRE | - | AGGG |
| TATA-box | TATA-box | TATA-box | core promoter element around -30 of transcription start |
| TATC-box | - | - | cis-acting element involved in gibberellin-responsiveness | TATCCCA |
| - | TCCC-motif | - | part of a light responsive element | TCTCCCT |
| TC-rich | TC-rich | TC-rich | cis-acting element involved in defense and stress responsiveness | ATTCTCTAAC |
| TCT-motif | TCT-motif | TCT-motif | part of a light responsive element | TCTTAC |
| TGA-element | - | - | auxin-responsive element | AACGAC |
| - | TGACG-motif | TGACG-motif | cis-acting regulatory element involved in the MeJA-responsiveness | TGACG |
| W box | W box | W box | - | TTGACC |