

Supplementary Material

1 Supplementary Tables

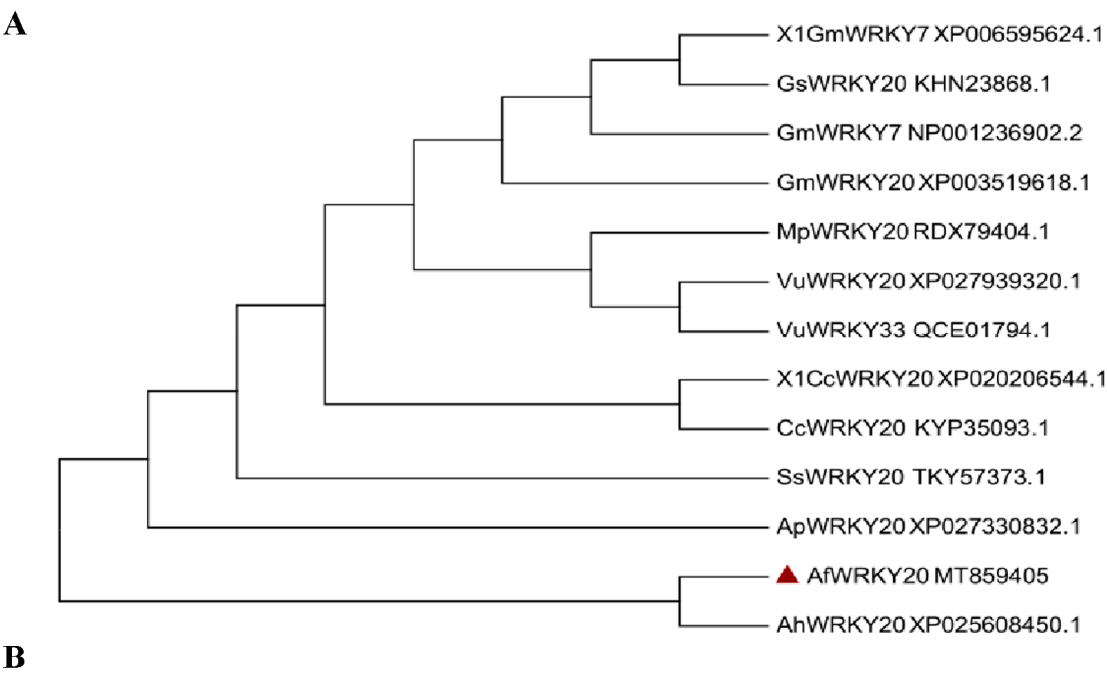
Table S1 Primers needed in the experiment

| Primer name | Primer Sequence (5'-3') | Function |
|------------------------|--|--|
| <i>AfWRKY20</i> -F1 | TCTAGACTATGAACGGAGCTACCACC | Gene Cloning |
| <i>AfWRKY20</i> -R1 | GTCGACATGGACCCGTTAGTATTCT | |
| <i>AfTubu.F</i> | ACAAGGCGGTAAAGGTTGGT | <i>Amorpha fruticosa</i> internal reference genes |
| <i>AfTubu.R</i> | GTTCTGGGCTTGGTTCCTT | |
| q <i>AfWRKY20</i> F | GAGTCCGACGTCGTTTTTGG | Real-time quantitative specific primers |
| q <i>AfWRKY20</i> R | TTCAACAGGAGCACTAGCCC | |
| W-box F1 | GGAACCTTGACCTTGACCTTAGGGCTGCAGGA ATTCTG | Probe primers for the W-box of EMSA |
| W-box R1 | CGAATTCCTGCAGCCCCTAAGGTCAAGG TCAAGTCC | |
| W-box F | GGGCCCCCCTCGAGGTCGACCGTTGAC CGTTGACCGAGTTGACTTTTTTAG | W-box probe primer for the Dual LUC activity |
| W-box R | CGCTCTAGAACTAGTGGATCCTAAAAAA GTCAACTCGGTCAACGGTCAACGC | |

| | | |
|-------------------------|-------------------------------------|-------------------------------------|
| mW-box F | GGGCCCCCCTCGAGGTCGACCGTAGAC | mW-box probe primer for dual |
| | GGTAGACGGAGTAGACGTTTTTAG | LUC activity |
| mW-box R | CGCTCTAGAACTAGTGGATCCTAAAAAC | |
| | GTCTACTCCGTCTACCGTCTACGC | |
| <i>AfWRKY20</i> | CGCTCTAGAACTAGTGGATCCATGAACG | Gene primers for Double LUC |
| -62-SK F | GAGCTACCACCAA | activity |
| <i>AfWRKY20</i> | GGTACCGGGCCCCCCTCGAGGTCGACT | |
| -62-SK R | CATGGACCCGTTAGTATTCTTC | |
| <i>NbActin</i> F | CTGCTGGAATTCACGAAACA | Tabacco internal |
| | | reference genes |
| <i>NbActin</i> R | GCCACCACCTTGATCTTCAT | |
| <i>NbSOD</i> F | CTCCTACCGTCGCCAAAT | Real-time quantitative |
| | | specific primers |
| <i>NbSOD</i> R | GCCCAACCAAGAGAACCC | |
| <i>NbCAT</i> F | AGGTACCGCTCATTACACCC | Real-time quantitative |
| | | specific primers |
| <i>NbCAT</i> R | AAGCAAGCTTTTGACCCAGA | |
| <i>NbPOD</i> F | TCCTGGAGTTGTTGCCTTGCTAG | Real-time quantitative |
| | | specific primers |

NbPOD R GCTTAGTCCTCTTGTCGGTTGCTA

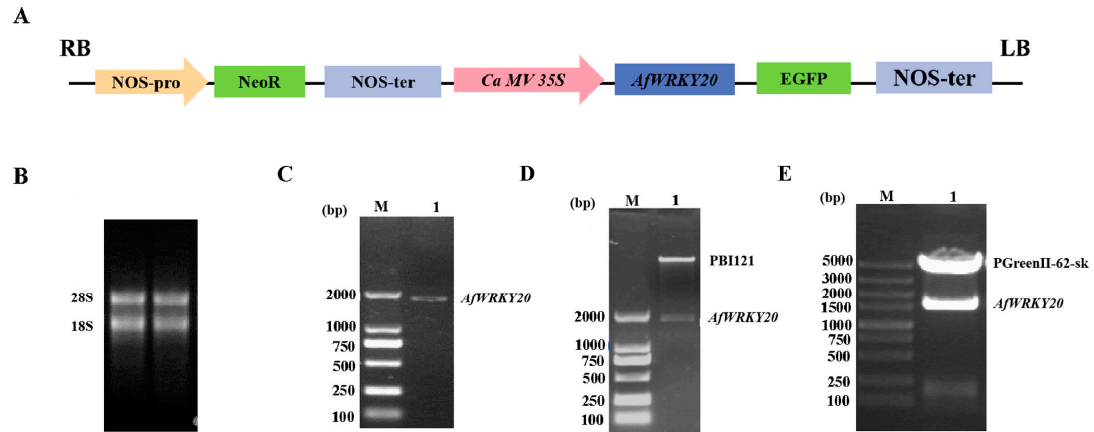
2 Supplementary Figures



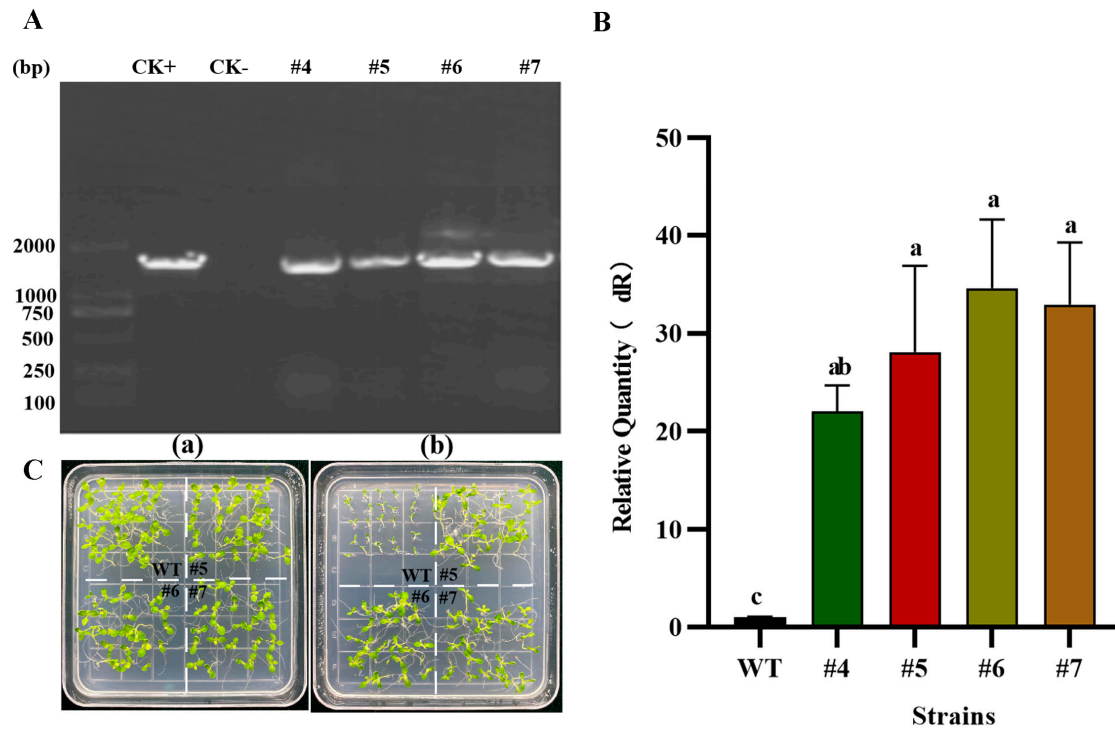
----- Plant-mPLoc Computation Result -----

| Query protein | Predicted location(s) |
|---------------------------------------|-----------------------|
| UEC49143.1 WRKY20 [Amorpha fruticosa] | Nucleus. |

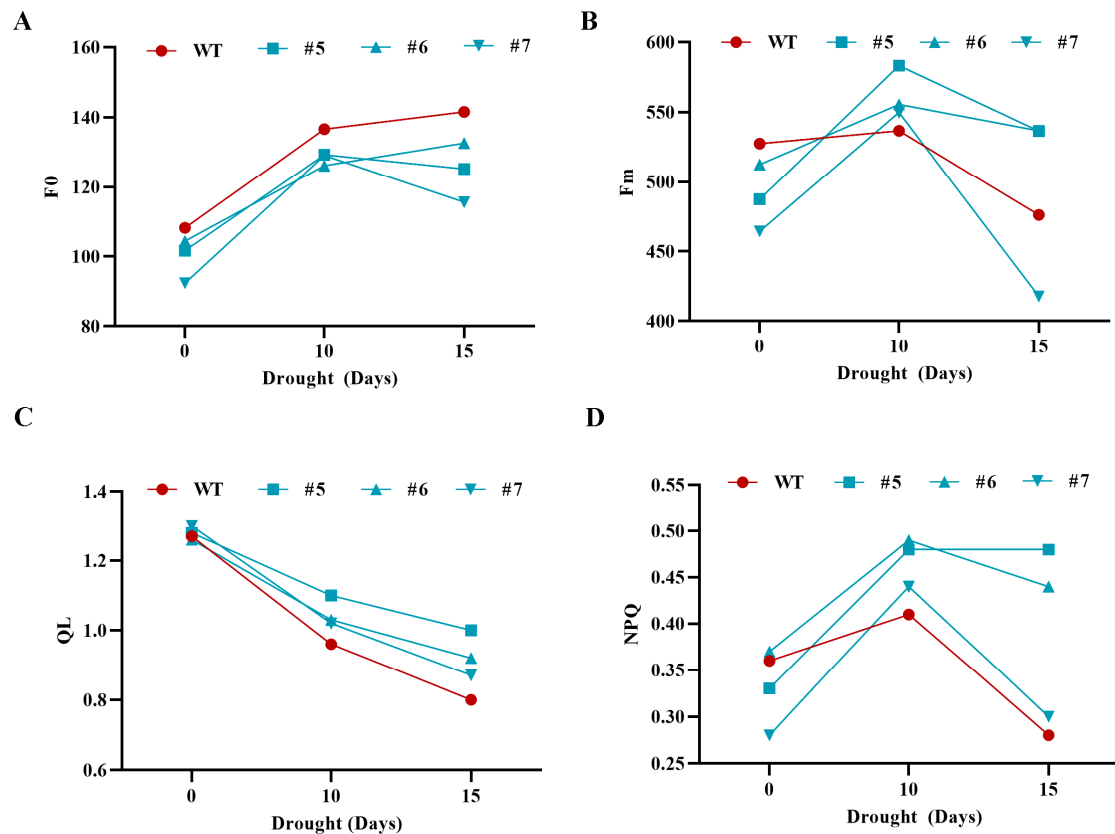
Supplementary Figure S1. (A) AfWRKY20 protein phylogenetic tree. Note: *Spatholobus suberectus*; *Glycine max*; *Cajanus cajan*; *Abrus precatorius*; *Mucuna pruriens*; *Vigna unguiculata*; *Arachis hypogaea*; *Glycine soja*. (B) Predicted subcellular localization of AfWRKY20 protein.



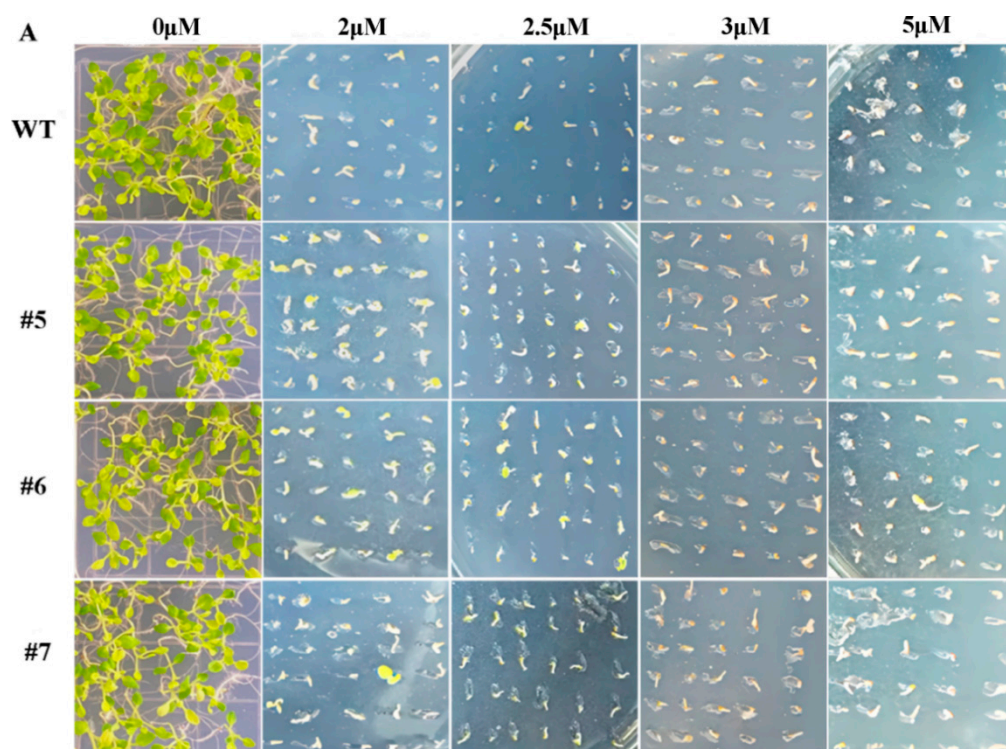
Supplementary Figure S2. (A) Diagram of pBI121-*AfWRKY20*-GFP. (B) Extraction of total RNA from *Amorpha fruticosa* leaves. (C) PCR amplification product electrophoresis detection. (D) Identification of pBI121::*AfWRKY20*::GFP digest. (E) Identification of pGreenII-62-sk::*AfWRKY20* digest.

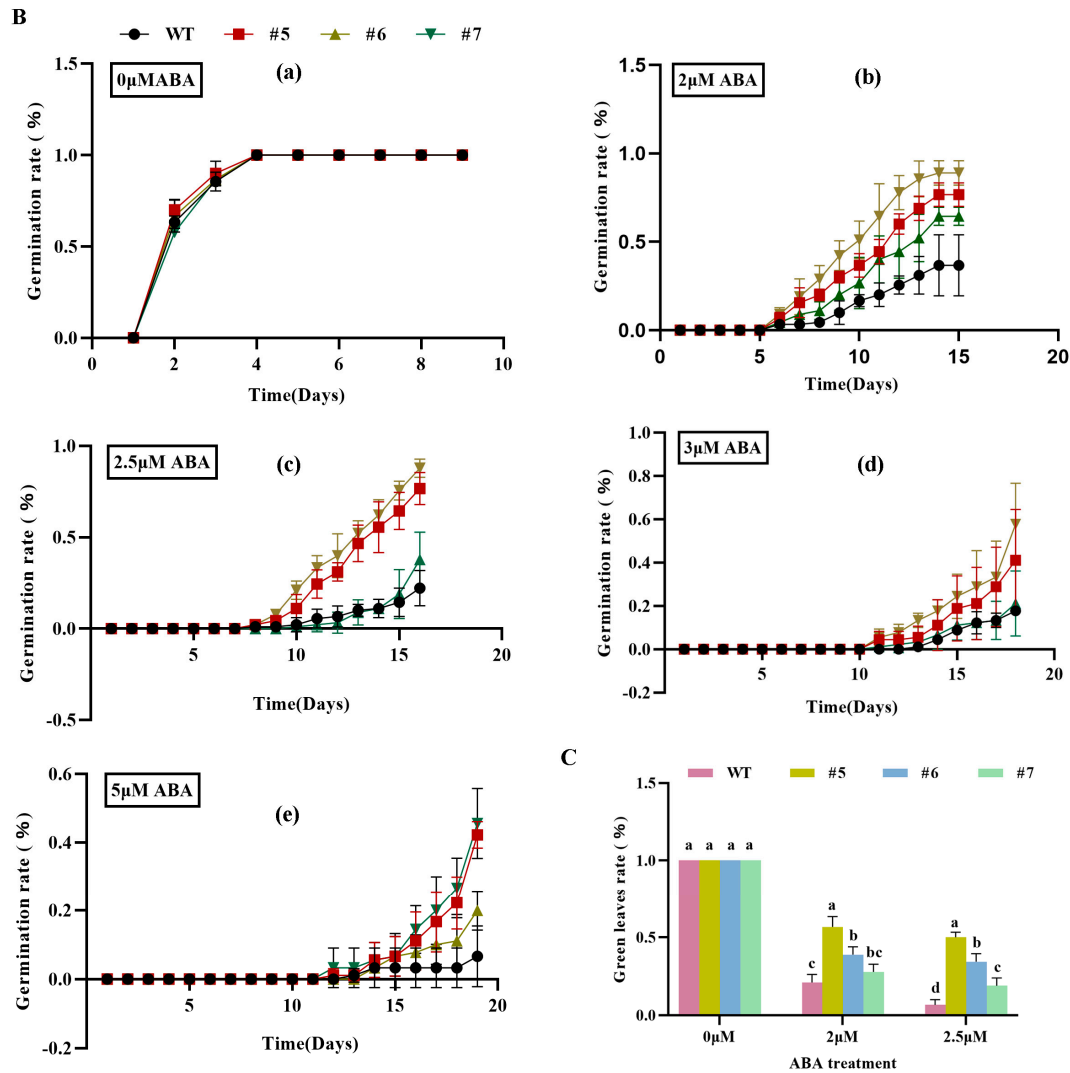


Supplementary Figure S3. (A) PCR identification of transgenic plants. (B) Relative expression analysis of *AfWRKY20* transgenic tobacco. Lowercase letters (a, b, c, etc.) represent statistical differences with $p < 0.05$. (C) (a): 1/2 MS medium; (b): Kana resistance screening of overexpressed *AfWRKY20* tobacco transformed seedlings.

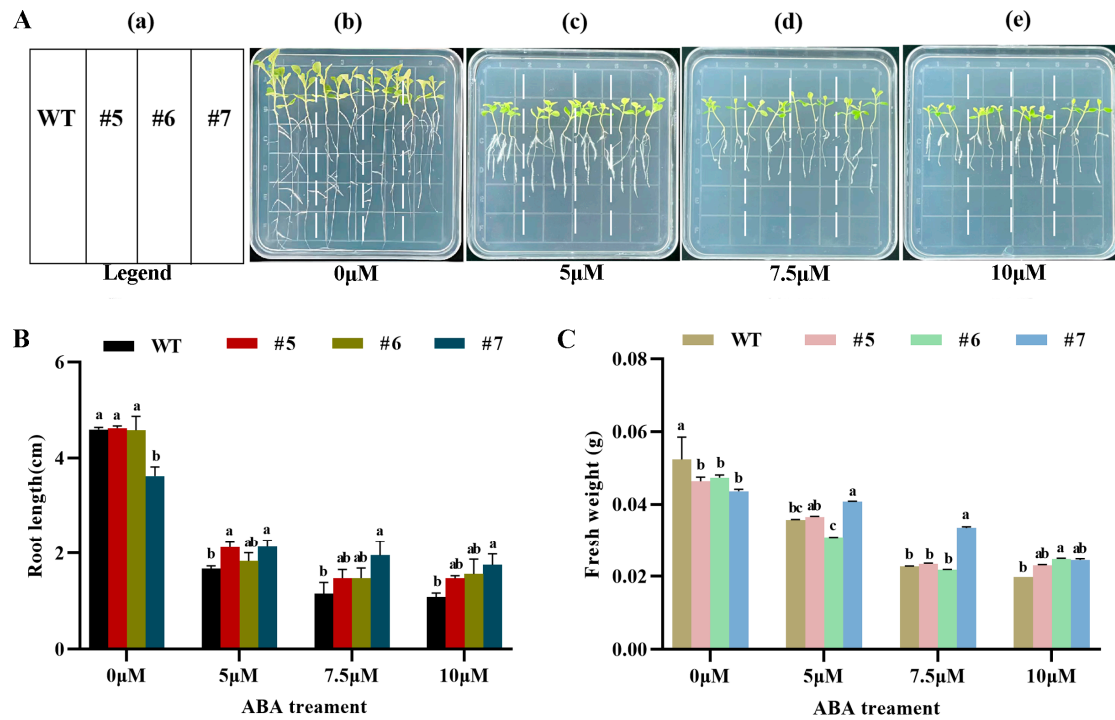


Supplementary Figure S4. Changes in chlorophyll fluorescence parameters of tobacco under natural drought stress for 15 days. (A) Variation of the minimum fluorescence yield (F0) in the absence of photosynthetic light. (B) Variation of maximum fluorescence yield (Fm) in the absence of photosynthetic light. (C) Variation of the proportion of open PSII reaction centers (QL). (D) Non-photochemical quenching (NPQ).





Supplementary Figure S5. Analysis of green leaf rate and germination rate of overexpression strain *AfWRKY20* under different concentrations of ABA stress. (A) Germination phenotype of ABA treatment. (B) Measurement of plant germination rate. (a–e) Trends of tobacco seed germination under different ABA stresses. (C) Measurement of plant green leaf rate. Error bars indicate standard errors of three biological replicates, which are significantly different at the $p < 0.05$ level. Lowercase letters (a, b, c, etc.) represent statistical differences with $p < 0.05$.



Supplementary Figure S6. Analysis of fresh weight and root length of overexpression strain *AfWRKY20* under different concentrations of ABA stress. WT: wild-type plants; #5, #6 and #7 are three different overexpression strains. (A) Phenotype of ABA stress. (a) Schematic diagram of tobacco placement. (b–e) Phenotypic map of tobacco after ABA stress at different concentrations. (B) Plant root length measurement data. (C) Plant fresh weight measurement data. Error lines indicate standard errors of three biological replicates, which are significantly different at the $p < 0.05$ level. Lowercase letters (a, b, c, etc.) represent statistical differences with $p < 0.05$.