# Genomic insights of the *WRKY* genes in Kenaf (*Hibiscus cannabinus* L.) reveal that *HcWRKY44* improves the plant's tolerance to the salinity stress

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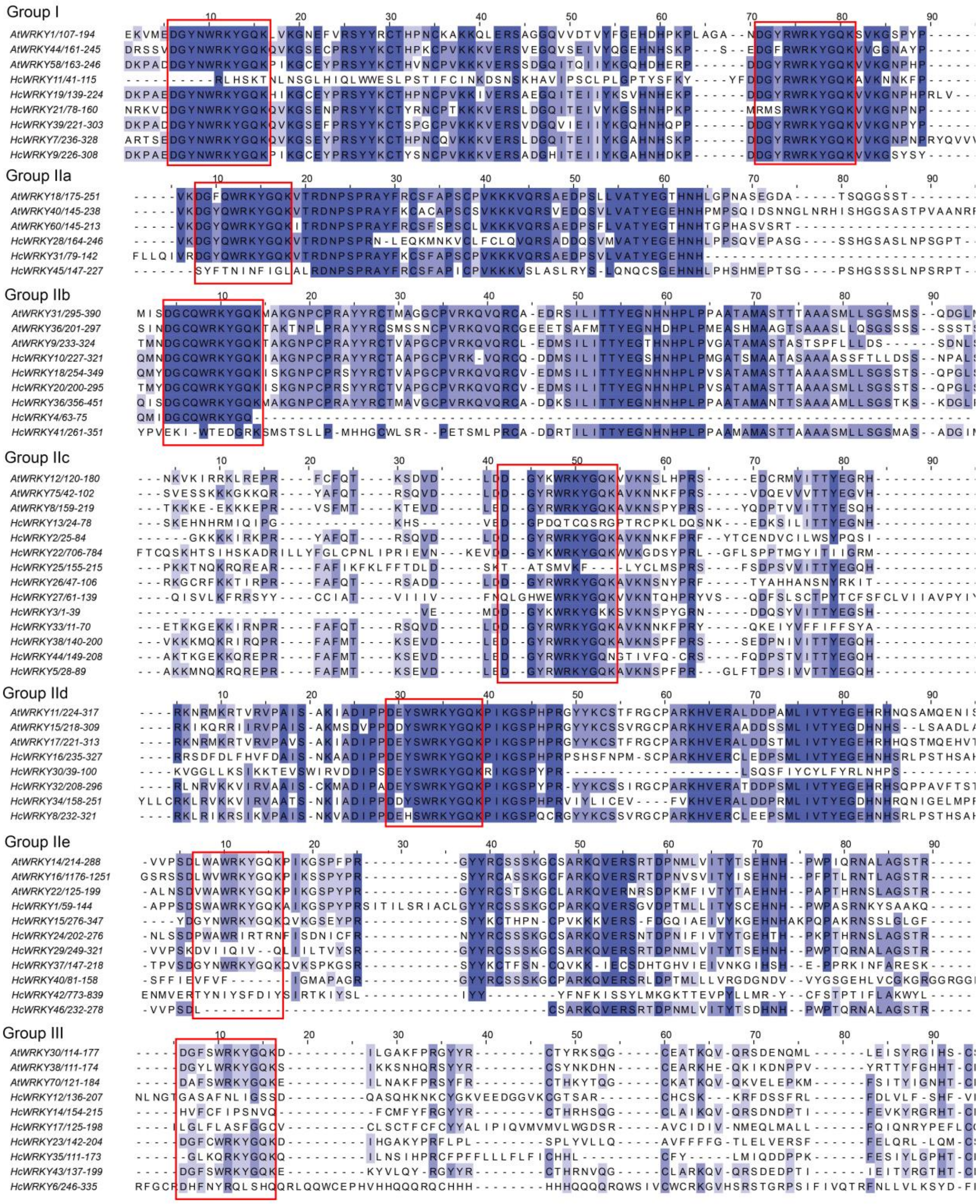
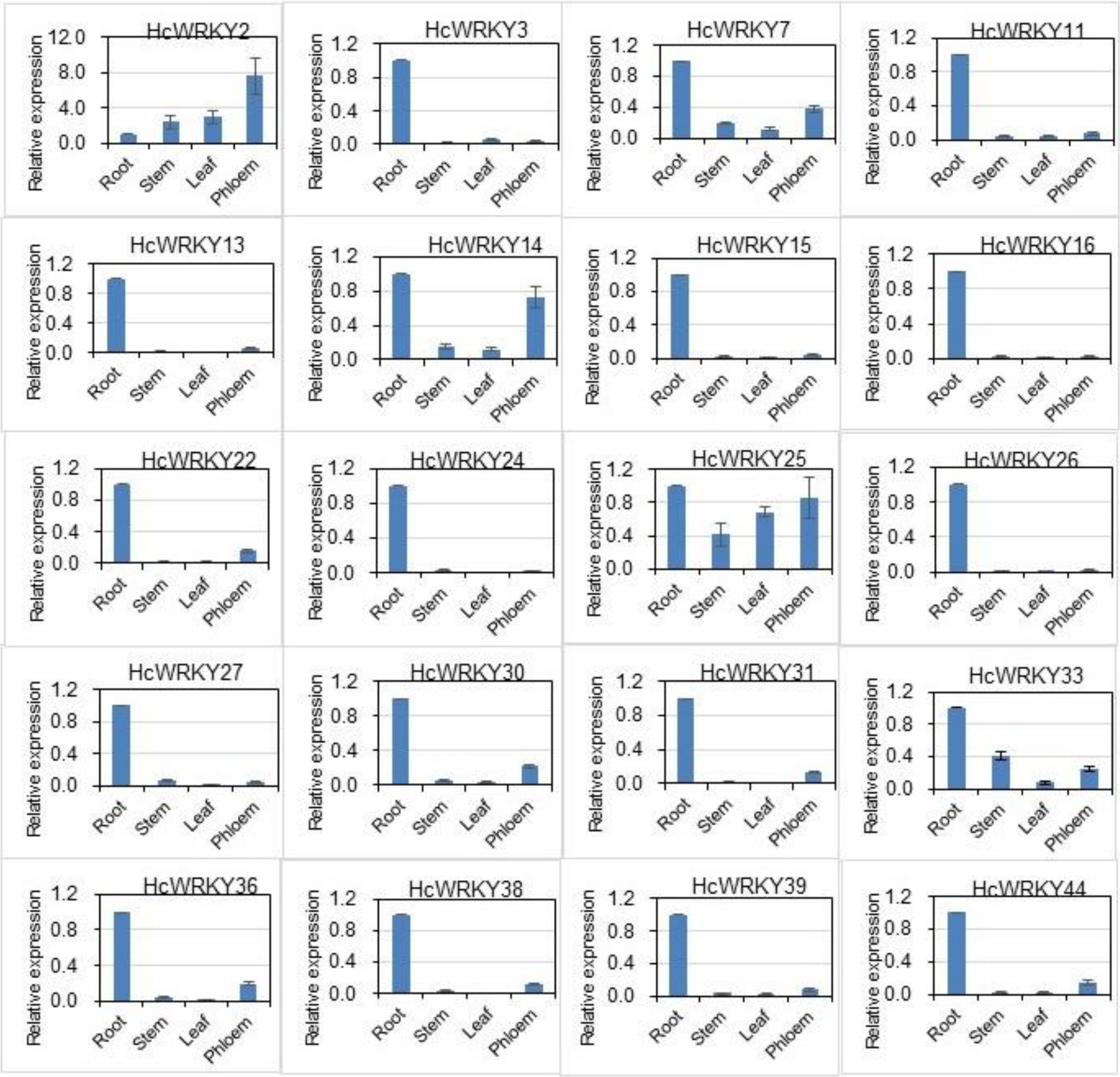
**Supplementary Figure S1. Growth state of kenaf variety Fuhong992 in the saline-alkali soil and its application for building materials.**

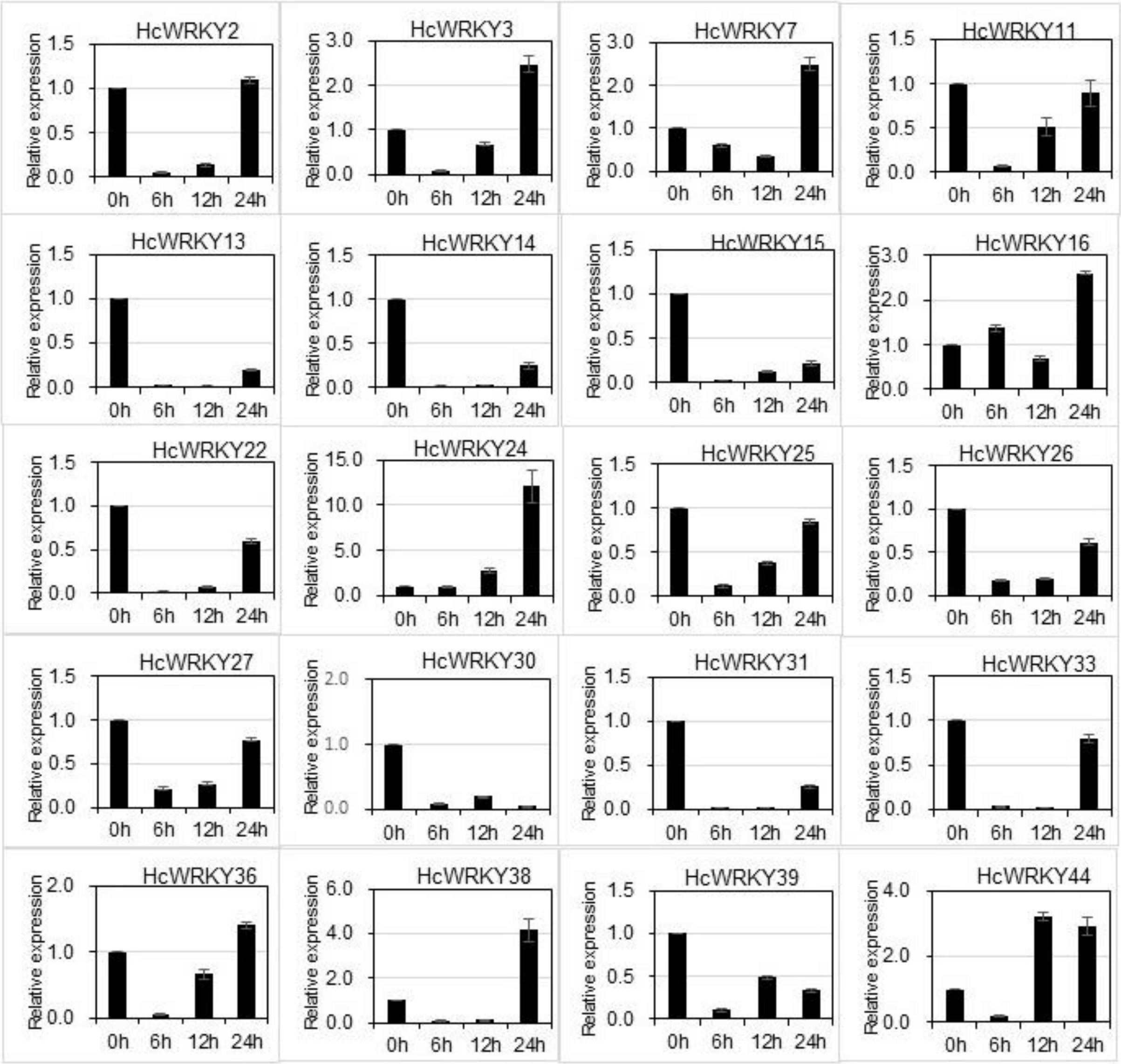
**Supplementary Figure S2. Multiple sequence alignment analysis of HcWRKY amino acids.** Multiple sequence alignment of deduced amino acid sequences of HcWRKY proteins with the representative WRKY proteins from *Arabidopsis*. Sequences were aligned using ClustalX. The typical WRKY domain is underlined and the completely conserved WRKYGQ(K)K amino acids were boxed in red.



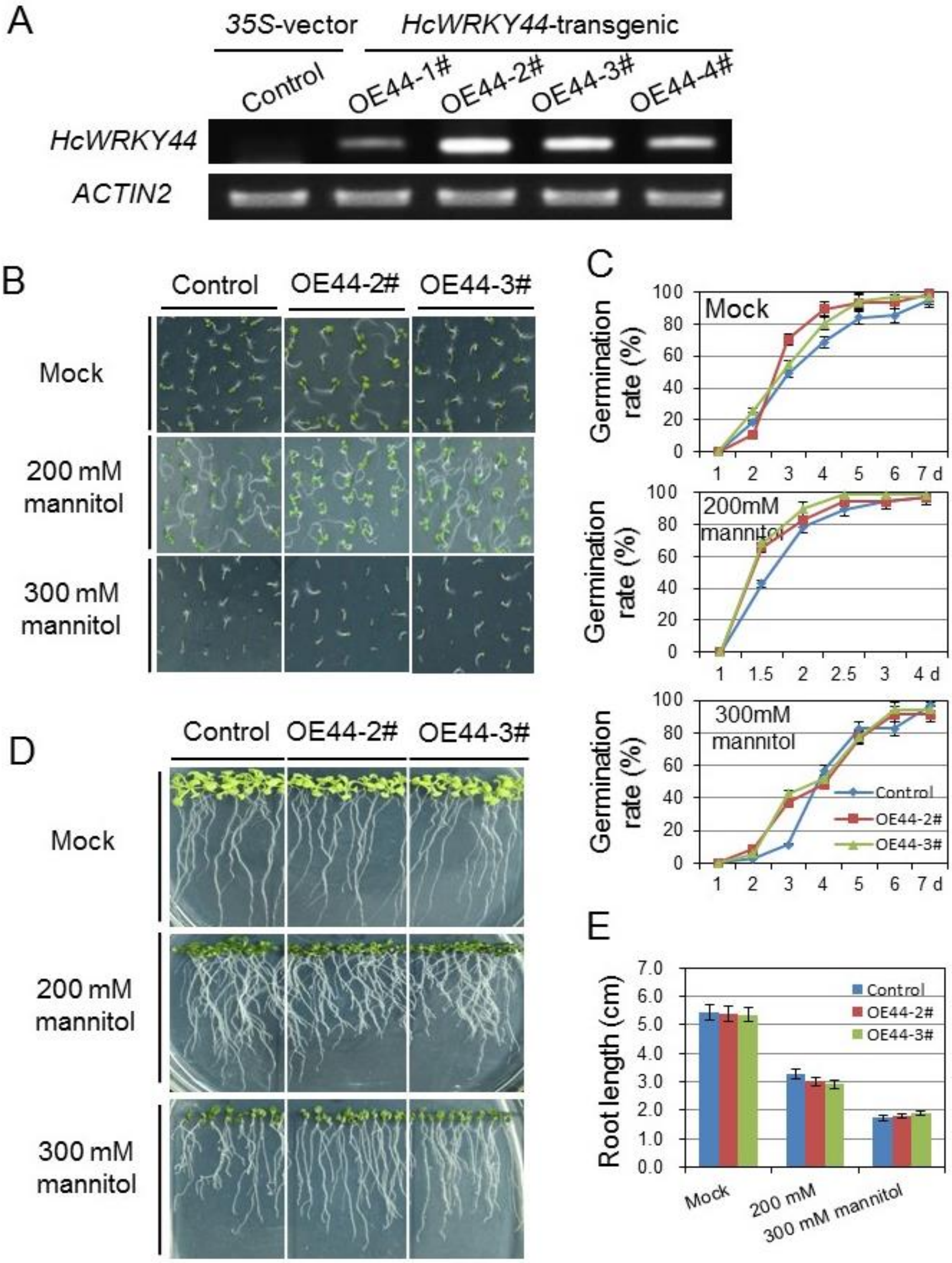
**Supplementary Figure S3. Expression profiles of 20 *HcWRKY* genes in different tissues.** 20 *HcWRKY* genes were cloned and selected for expression analysis in different tissues. Roots, stems, leaves and phloem of four-week-old kenaf seedlings were sampled and harvested for expression analysis by qRT-PCR. The *18S rRNA* and *TUBα* gene was used as the normalization factors for qRT-PCR analysis. Each assay was replicated three times.



**Supplementary Figure S4. Expression profiles of 20 *HcWRKY* genes under drought stress.** 20 *HcWRKY* genes were cloned and selected for expression analysis under drought stress, mimicked by 300 mM PEG solution irrigation. After treatment, leaves were harvested and used for mRNA transcripts analysis by qRT-PCR. The *18S rRNA* and *TUBα* gene was used as the standard control to normalize the qRT-PCR results. Each assay was replicated three times.



**Supplementary Figure S5. Overexpression of *HcWRKY44* in *Arabidopsis* did not affect the tolerance to drought stress.** (A) Semi-quantitative RT-PCR analysis of *HcWRKY44* transcript levels in homozygous 35S::*HcWRKY44* lines. *Actin2* was used as a control. (B and C) The seed germination rate on 1/2 MS with or without mannitol in different *HcWRKY44* transgenic lines (OE44-2# and OE44-3#) and control lines. Photographs were taken at 7 d after sowing. (D and E) Five-day-old seedlings grown on 1/2 MS were transferred to new plates supplemented with 0, 200, and 300 mM mannitol. The root growth of transgenic and control seedlings grown on mannitol supplemented plates were photographed and measured. All values are means (±SD) from three independent experiments. ***P < 0.01 by Student’s t-test.



**Supplementary Table S1.** The primers used in this study.

| **Name** | **Forward Primer** | **Reverse Primer** | **Purpose** |
|---|---|---|---|
| *HcWRKY2* | GTTTAACGTGTGGTGCATCG | AGACAGGGCAAGATTCCAGA | qRT-PCR |
| *HcWRKY3* | CTCCACCGGTATCCATCATC | CATCTCAGGGCCAATTCAGT | qRT-PCR |
| *HcWRKY7* | CCTGGGTAGCCCTATCATCA | TCAACTGCACAAGGCAAATC | qRT-PCR |
| *HcWRKY11* | AGCATGTCTCGGTTTTCTCG | ACAATGGGATGCTGGGATTA | qRT-PCR |
| *HcWRKY13* | TTCGCTCCCCACAAGTTATC | ATTCGGATCAGAAGCAATGG | qRT-PCR |
| *HcWRKY14* | TATTTCCGCCAGCAATATCC | ATTGGAATGGTTTCGTGCTC | qRT-PCR |
| *HcWRKY15* | ATCTGATGGAACCGGTCAAG | GCATTCCCCACTAAGTCCAA | qRT-PCR |
| *HcWRKY16* | GCTGAAATTCAAGCCTTTGC | TTCCTGGAAAGTCCCAGTTG | qRT-PCR |
| *HcWRKY22* | CATCATTGGCAGGATGTCAG | GACCAAGAGGAGGCATGGTA | qRT-PCR |
| *HcWRKY24* | AAAGGATCCTCACGGGAACT | TAGGAATGGCTGAGGATTGG | qRT-PCR |
| *HcWRKY25* | GATCGACCTCGCTCTTTGTC | TCGATTTCATCAGCATCGAG | qRT-PCR |
| *HcWRKY26* | CCATCAGCAGCAGCAACTTA | CGACTCCTTGTTTGGAAAGC | qRT-PCR |
| *HcWRKY27* | ATCCTCAGCCAGTCTCTCCA | TCAAGACCAAGAGCGATGTG | qRT-PCR |
| *HcWRKY30* | CAACATAAACCCCAGCGACT | CCGGAGGAAGTACAAAACGA | qRT-PCR |
| *HcWRKY31* | GAAGAGGAAAGCCGAGTGTG | CCTTAGGCTTTTTGCACGAG | qRT-PCR |
| *HcWRKY33* | GATCGTGAGAAGGGGAATGA | AGTACGGCCAGAAAGTGGTG | qRT-PCR |
| *HcWRKY36* | CCGGAGAAATGGCTAAAACA | TATGGTGGGGAAGGCAGATA | qRT-PCR |
| *HcWRKY38* | TCCATCTTCCTCGTTGAACC | TGATAACCGCCTCCATTTTC | qRT-PCR |
| *HcWRKY39* | TCAGGGAAATTCCGAATCAG | CACCAGCTTCCTCACTGTCA | qRT-PCR |
| *HcWRKY44* | TCTCAGTGTCGTCGGAAGTG | GGCAGAAGGCTGTGAAGAAC | qRT-PCR |
| *RD22* | GGTTCGGAAGAAGCGGAG | GAAACAGCCCTGACGTGATAT | qRT-PCR |
| *COR47* | GGAGTACAAGAACAACGTTCCCGA | TGTCGTCGCTGGTGATTCCTCT | qRT-PCR |
| *COR15A* | GGCCACAAAGAAAGCTTCAG | CTTGTTTGCGGCTTCTTTTC | qRT-PCR |
| *KIN1* | AACAAGAATGCCTTCCAAGC | CGCATCCGATACACTCTTTCC | qRT-PCR |
| *P5CS* | GCGCATAGTTTCTGATGCAA | TGCAACTTCGTGATCCTCTG | qRT-PCR |
| *ABI1* | AGAGTGTGCCTTTGTATGGTTTTA | CATCCTCTCTCTACAATAGTTCGCT | qRT-PCR |
| *ABI2* | GATGGAAGATTCTGTCTCAACGATT | GTTTCTCCTTCACTATCTCCTCCG | qRT-PCR |
| *ABI5* | CAATAAGAGAGGGATAGCGAACGAG | CGTCCATTGCTGTCTCCTCCA | qRT-PCR |
| *ABF4* | AACAACTTAGGAGGTGGTGGTC | CTTCAGGAGTTCATCCATGTTC | qRT-PCR |
| *ACTIN2* | TCAGATGCCCAGAAGTGTGTT | CCGTACAGATCCTTCCTGATAT | qRT-PCR |
| *RD29B* | ACGCATAAAGGTGGAGAAGC | TCTTGCCGGAGAATTCTTGT | qRT-PCR |
| *DREB2A* | AAGGGTCGAAGAAGGGTTGT | CGAGCCAAAGGACCATACAT | qRT-PCR |
| *STZ* | CTAGTAGCGTGTCCAACTCCG | TTTGACCGGAAAGTCAAACCG | qRT-PCR |
| *SOS1* | GTGAAGCAATCAAGCGGAAA | TGCGAAGAAGGCGTAGAACA | qRT-PCR |
| *AHA1* | CACAAACATTTACCGAAAACCA | CAAATTTGCAAAGCTCATATCG | qRT-PCR |
| *AHA2* | TGACTGATCTTCGATCCTCTCA | GAGAATGTGCATGTGCCAAA | qRT-PCR |
| *HKT1* | GATTTGTCCCCACGAATGAGA | CAAAACCAAGAAGCAAGGGAAC | qRT-PCR |

**Supplementary Table S2.** Inference of duplication time of 6 synteny *WRKY* gene pairs in kenaf.

| **Paralogous gene pairs** | **Ka** | **Ks** | **Ka/Ks** | **MYA (million years ago)** |
|---|---|---|---|---|
| *HcWRKY1/HcWRKY40* | 0.935404 | 1.56222 | 0.598766 | 120.171 |
| *HcWRKY5/HcWRKY17* | 0.584093 | 2.15432 | 0.271127 | 165.717 |
| *HcWRKY8/HcWRKY16* | 0.118127 | 0.287554 | 0.410798 | 22.119 |
| *HcWRKY18/HcWRKY20* | 0.168774 | 0.161009 | 1.04823 | 12.385 |
| *HcWRKY28/HcWRKY45* | 0.321108 | 0.543086 | 0.591266 | 41.776 |
| *HcWRKY29/HcWRKY46* | 0.162325 | 0.331053 | 0.49033 | 25.466 |

**Supplementary Table S3.** 16 syntenic orthologous gene pairs were identified between *H. cannabinus* and *A. thaliana.*

| **Gene1 ID** | **Gene1 name** | **Gene1 ID** | **Gene2 name** |
|---|---|---|---|
| *Hca.06G0019130-mRNA-1* | *HcWRKY16* | *AT3G04670.1* | *AtWRKY39* |
| *Hca.09G0003050-mRNA-1* | *HcWRKY1* | *AT1G29280.1* | *AtWRKY65* |
| *Hca.10G0029380-mRNA-1* | *HcWRKY39* | *AT1G13960.1* | *AtWRKY4* |
| *Hca.10G0029380-mRNA-1* | *HcWRKY39* | *AT2G03340.1* | *AtWRKY3* |
| *Hca.10G0029300-mRNA-1* | *HcWRKY38* | *AT1G69310.1* | *AtWRKY57* |
| *Hca.06G0041200-mRNA-1* | *HcWRKY18* | *AT5G15130.1* | *AtWRKY72* |
| *Hca.06G0008140-mRNA-1* | *HcWRKY15* | *AT2G37260.1* | *AtWRKY44* |
| *Hca.17G0025330-mRNA-1* | *HcWRKY24* | *AT5G52830.1* | *AtWRKY27* |
| *Hca.17G0006480-mRNA-1* | *HcWRKY21* | *AT2G38470.1* | *AtWRKY33* |
| *Hca.07G0034910-mRNA-1* | *HcWRKY29* | *AT1G30650.1* | *AtWRKY14* |
| *Hca.07G0034360-mRNA-1* | *HcWRKY28* | *AT1G80840.1* | *AtWRKY40* |
| *Hca.03G0036480-mRNA-1* | *HcWRKY44* | *AT1G29860.1* | *AtWRKY71* |
| *Hca.02G0001850-mRNA-1* | *HcWRKY5* | *AT2G47260.1* | *AtWRKY23* |
| *Hca.02G0001850-mRNA-1* | *HcWRKY5* | *AT3G62340.1* | *AtWRKY68* |
| *Hca.06G0030610-mRNA-1* | *HcWRKY17* | *AT2G47260.1* | *AtWRKY23* |
| *Hca.04G0029560-mRNA-1* | *HcWRKY14* | *AT2G46400.1* | *AtWRKY46* |

**Supplementary Table S4.** 64 syntenic orthologous gene pairs were identified between *H. cannabinus* and *G. hirsutum*.

| Gene1 ID | Gene1 name | Gene2 ID | Gene2 name |
|---|---|---|---|
| Hca.18G0000330-mRNA-1 | HcWRKY32 | KJB06621 | GrWRKY17 |
| Hca.02G0034140-mRNA-1 | HcWRKY8 | KJB44894 | GrWRKY74 |
| Hca.01G0010340-mRNA-1 | HcWRKY34 | KJB58556 | GrWRKY7 |
| Hca.06G0019130-mRNA-1 | HcWRKY16 | KJB26553 | GrWRKY74 |
| Hca.06G0019130-mRNA-1 | HcWRKY16 | KJB44894 | GrWRKY74 |
| Hca.09G0003050-mRNA-1 | HcWRKY1 | KJB66852 | GrWRKY65 |
| Hca.09G0003050-mRNA-1 | HcWRKY1 | KJB69691 | GrWRKY65 |
| Hca.09G0003050-mRNA-1 | HcWRKY1 | KJB57301 | GrWRKY65 |
| Hca.10G0029380-mRNA-1 | HcWRKY39 | KJB75675 | GrWRKY3 |
| Hca.10G0029380-mRNA-1 | HcWRKY39 | KJB78251 | GrWRKY4 |
| Hca.10G0029380-mRNA-1 | HcWRKY39 | KJB80287 | GrWRKY3 |
| Hca.10G0029380-mRNA-1 | HcWRKY39 | KJB33491 | GrWRKY3 |
| Hca.07G0045110-mRNA-1 | HcWRKY31 | KJB07689 | GrWRKY18 |
| Hca.07G0045110-mRNA-1 | HcWRKY31 | KJB63352 | GrWRKY40 |
| Hca.07G0045110-mRNA-1 | HcWRKY31 | KJB55078 | GrWRKY40 |
| Hca.01G0053260-mRNA-1 | HcWRKY36 | KJB09521 | GrWRKY47 |
| Hca.01G0053260-mRNA-1 | HcWRKY36 | KJB49704 | GrWRKY47 |
| Hca.10G0029300-mRNA-1 | HcWRKY38 | KJB80329 | GrWRKY57 |
| Hca.10G0029300-mRNA-1 | HcWRKY38 | KJB30447 | GrWRKY57 |
| Hca.10G0029300-mRNA-1 | HcWRKY38 | KJB50588 | GrWRKY57 |
| Hca.06G0041200-mRNA-1 | HcWRKY18 | KJB75770 | GrWRKY72 |
| Hca.06G0041200-mRNA-1 | HcWRKY18 | KJB33673 | GrWRKY72 |
| Hca.05G0017730-mRNA-1 | HcWRKY9 | KJB75675 | GrWRKY3 |
| Hca.05G0017730-mRNA-1 | HcWRKY9 | KJB33491 | GrWRKY3 |
| Hca.06G0008140-mRNA-1 | HcWRKY15 | KJB62524 | GrWRKY44 |
| Hca.17G0025330-mRNA-1 | HcWRKY24 | KJB52708 | GrWRKY27 |
| Hca.17G0006480-mRNA-1 | HcWRKY21 | KJB77081 | GrWRKY33 |
| Hca.07G0034910-mRNA-1 | HcWRKY29 | KJB56367 | GrWRKY14 |
| Hca.06G0041680-mRNA-1 | HcWRKY19 | KJB75675 | GrWRKY3 |
| Hca.06G0041680-mRNA-1 | HcWRKY19 | KJB33491 | GrWRKY3 |
| Hca.07G0034360-mRNA-1 | HcWRKY28 | KJB63352 | GrWRKY40 |
| Hca.07G0034360-mRNA-1 | HcWRKY28 | KJB68021 | GrWRKY40 |
| Hca.07G0034360-mRNA-1 | HcWRKY28 | KJB56530 | GrWRKY40 |
| Hca.03G0036480-mRNA-1 | HcWRKY44 | KJB57293 | GrWRKY71 |
| Hca.02G0001850-mRNA-1 | HcWRKY5 | KJB24423 | GrWRKY23 |
| Hca.02G0001850-mRNA-1 | HcWRKY5 | KJB41782 | GrWRKY23 |
| Hca.02G0001850-mRNA-1 | HcWRKY5 | KJB49782 | GrWRKY23 |
| Hca.02G0005160-mRNA-1 | HcWRKY6 | KJB10662 | GrWRKY31 |
| Hca.02G0005160-mRNA-1 | HcWRKY6 | KJB42875 | GrWRKY31 |
| Hca.04G0026330-mRNA-1 | HcWRKY12 | KJB25976 | GrWRKY21 |

| Gene1 ID | Gene1 name | Gene2 ID | Gene2 name |
|---|---|---|---|
| Hca.14G0009150-mRNA-1 | HcWRKY40 | KJB66852 | GrWRKY65 |
| Hca.04G0026330-mRNA-1 | HcWRKY12 | KJB42219 | GrWRKY21 |
| Hca.14G0009150-mRNA-1 | HcWRKY40 | KJB69691 | GrWRKY65 |
| Hca.14G0009150-mRNA-1 | HcWRKY40 | KJB57301 | GrWRKY65 |
| Hca.08G0021600-mRNA-1 | HcWRKY25 | KJB24423 | GrWRKY23 |
| Hca.06G0030610-mRNA-1 | HcWRKY17 | KJB24423 | GrWRKY23 |
| Hca.06G0030610-mRNA-1 | HcWRKY17 | KJB41782 | GrWRKY23 |
| Hca.06G0030610-mRNA-1 | HcWRKY17 | KJB49782 | GrWRKY23 |
| Hca.09G0009010-mRNA-1 | HcWRKY2 | KJB11703 | GrWRKY75 |
| Hca.09G0009010-mRNA-1 | HcWRKY2 | KJB70671 | GrWRKY75 |
| Hca.08G0022540-mRNA-1 | HcWRKY26 | KJB24642 | GrWRKY43 |
| Hca.18G0023790-mRNA-1 | HcWRKY33 | KJB15502 | GrWRKY75 |
| Hca.18G0023790-mRNA-1 | HcWRKY33 | KJB30860 | GrWRKY75 |
| Hca.10G0002810-mRNA-1 | HcWRKY37 | KJB06578 | GrWRKY32 |
| Hca.03G0042970-mRNA-1 | HcWRKY45 | KJB56530 | GrWRKY40 |
| Hca.14G0010950-mRNA-1 | HcWRKY41 | KJB67258 | GrWRKY31 |
| Hca.14G0010950-mRNA-1 | HcWRKY41 | KJB69258 | GrWRKY6 |
| Hca.04G0028600-mRNA-1 | HcWRKY13 | KJB41730 | GrWRKY47 |
| Hca.04G0029560-mRNA-1 | HcWRKY14 | KJB09677 | GrWRKY46 |
| Hca.04G0029560-mRNA-1 | HcWRKY14 | KJB09677 | GrWRKY46 |
| Hca.04G0029560-mRNA-1 | HcWRKY14 | KJB41500 | GrWRKY46 |
| Hca.17G0024970-mRNA-1 | HcWRKY23 | KJB52625 | GrWRKY30 |
| Hca.03G0043540-mRNA-1 | HcWRKY46 | KJB56367 | GrWRKY14 |
| Hca.01G0035190-mRNA-1 | HcWRKY35 | KJB27670 | GrWRKY70 |

Supplementary Table S4 (continued).