

Supplementary materials

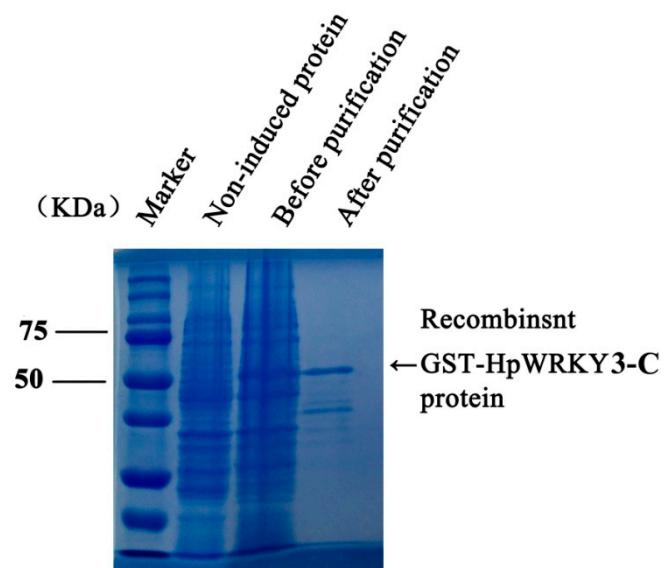


Figure S1. SDS-PAGE gel stained with Coomassie blue demonstrating affinity purification of the recombinant GST-WRKY3-C terminal.

Table S1 Main regulatory motifs found within the *HpINV2* promoter

Factor or site name	Signal sequence	Site	Function
AE-box	AGAAACTT	197(+)	part of a module for light response
Box 4	ATTAAT	720(+)	part of a conserved DNA module involved in light responsiveness
CAAT-box	CAAT	28(+),42(+),392(-),694(+),730(-),777(+),811(-), 1091(+),1099(-),1164(+)	common cis-acting element in promoter and enhancer regions
	CAAAT	163(+),391(-),399(-),470(+),668(-),1080(-), 1088(-),1208(-)	
	CCAAT	1073(+), 1098(-)	
GC-motif	CCCCCG	115(+)	enhancer-like element involved in anoxic specific inducibility
GCN4_motif	TGAGTCA	1004(-)	cis-regulatory element involved in endosperm expression
Myb-binding site	CAACAG	1011(-)	MYB binding site
MYC	CATTG	1086(+),1208(+)	MYC recognition elements
TATA-box	TACAAAAA	191(-),366(-),445(-)	core promoter element around -30 of transcription start
	TATA	293(-),303(-),330(-),341(-),375(-),399(-),409(-), 439(-),456(-),471(-),496(-),504(-),601(-),634(+), 690(+),741(+),795(+),800(+),831(+),933(+)	
	TATTTAAA	101(+),559(-)	
TCT-motif	TCTTAC	312(-),856(-)	part of a light responsive element
W box	TTGACC	1123(-)	WRKY binding site
	TTGACT	581(+)	

Table S2 Main regulatory motifs found within the *HpSuSy1* promoter

Factor or site name	Signal sequence	Site	Function
ARE	AAACCA	186(-),809(+)	cis-acting regulatory element essential for the anaerobic induction
		809(+)	
ATC-motif	AGTAATCT	1095(-)	part of a conserved DNA module involved in light responsiveness
CAAT-box	CAAT	68(-),72(-),206(+),424(+),494(-),921(-),1081(+),1151(-), 1174(+),1267(-),1269(+),1280(-),1374(-),1404(-),1415(-), 1468(-),1470(+),1562(-)	common cis-acting element in promoter and enhancer regions
	CAAAT	160(-),263(+),517(+),601(-),643(-),673(+),678(+),734(+), 775(+),788(+),1263(+),1360(-),1390(-)	
CAT-box	GCCACT	153(+),1305(-)	cis-acting regulatory element related to meristem expression
CGTCA-motif	CGTCA	694(-)	cis-acting regulatory element involved in the MeJA-responsiveness
ERE	ATTCATA	1231(-)	Ethylene-responsive element
GATA-motif	AAGGATAAGG	703(-),1578(+)	part of a light responsive element
GC-motif	CCCCCG	34(+)	enhancer-like element involved in anoxic specific inducibility
GT1-motif	GGTTAA	394(+)	light responsive element
MBS	CAACTG	1488(-)	MYB binding site involved in drought-inducibility
MYB	CAACCA	847(+)	MYB binding site
MYC	CATTG	161(+),734(-),788(-),1263(-),1269(+),1470(+)	MYC recognition elements
TATA-box	TACAAAA	512(+),813(+),905(+),1479(+)	core promoter element around -30 of transcription start
	TATA	89(-),279(-),332(-),352(-),504(-),531(-),542(-),577(-), 595(-),994(+),1028(+),1213(+),1440(+),1515(+),1594(+)	
TCA-element	CCATCTTTT	762(-)	cis-acting element involved in salicylic acid responsiveness
TCT-motif	TCTTAC	365(-)	part of a light responsive element
TGA-element	AACGAC	940(+)	auxin-responsive element
W box	TTGACC	1414(+)	WRKY binding site
	TTGACT	1485(+),1403(+)	
WUN-motif	AAATTCCT	1194(+)	wound-responsive element

Table S3 List of primers used in this study.

Assay	Primer sequence	Restriction Site
RT-qPCR	<i>HpINV1-qPCR-F:</i> TCAATCCTCGCCATCGAACTGC <i>HpINV1-qPCR-R:</i> GGACCACGCCCATTTTGTCA <i>HpINV2-qPCR-F:</i> ATGGTCACTGTACCAAGGG <i>HpINV2-qPCR-R:</i> GCCATCTGGCAAGATCGTAGC <i>HpSuSy1-qPCR-F:</i> AGGTATGTGGCTCAGGGGAAAG <i>HpSuSy1-qPCR-R:</i> GCTATGGCAACGAAGGGAGG <i>HpSuSy2-qPCR-F:</i> ATAGGCAATGGGTTGAGTTCC <i>HpSuSy2-qPCR-R:</i> TCAAGAGCCTCAGGTTCTGAA <i>HpWRKY3-qPCR-F:</i> CCTTTATCTCTGAGTCACGCC <i>HpWRKY3-qPCR-R:</i> ACAATCATGGAAAGAAGGCTGTG	
Promoter and Gene isolation	<i>HpINV2 PRO-SP1:</i> TTGAGCATATCATTGTCCAAGCAAACCTC <i>HpINV2 PRO-SP2:</i> CCGCCGGAGACTCCCTGCCGTAGGATGA <i>HpINV2 PRO-SP3:</i> GTTGGGCTGGGTCGATNNNNNNNNCCGTGG <i>HpSuSy1 PRO-SP1:</i> CTATGGCACGAAAGGGAGGGACGACGATTG <i>HpSuSy1 PRO-SP2:</i> TCATCGCGATCACCTTCAAGTTCATCAAT <i>HpSuSy1 PRO-SP3:</i> ACAGATGAGCAGAGAGAGAGNNNNNNNNCTTCT <i>HpINV2 PRO-F:</i> AGTTGGGAGTGGAGAGGG <i>HpINV2 PRO-R:</i> CATTGAGGAAGAGAAGAGTTTG <i>HpSuSy1 PRO-F:</i> ATATAACTTGTCTGAGAAGGAAAGGG <i>HpSuSy1 PRO-R:</i> CATTGGAGATGGACGGTTCTAGA <i>HpWRKY3-F:</i> ATGCCGAAAACGATGCCGTG <i>HpWRKY3-R:</i> CTATGTTATTCTCTGTGTTAAATGGAG	
Yeast One Hybrid	<i>HpINV2 PRO-pAbAi-F:</i> AATTGAAAAGCTTGAATTGAGCTCCCTCTGCTAACGAATTACAAGGTCAA <i>HpINV2 PRO-pAbAi-R:</i> ACAGAGCACATGCTCGAGGTCGACAATTAAAGAGTTGATTGATT <i>HpSuSy1 PRO-pAbAi-F:</i> AATTGAAAAGCTTGAATTGAGCTAACATAACTAAATGATAAAAGAAAATATC <i>HpSuSy1 PRO-pAbAi-R:</i> ACAGAGCACATGCCCTCGAGGTCGACCAATGGCAAGAAAAGTGTCTAGAGGTT <i>HpWRKY3-pGADT7-F:</i> GGAGGCCATTGAAATTATGCCGAAACAGATGCCGTG <i>HpWRKY3-pGADT7-R:</i> CGAGCTCGATGGATCCCTATGTTATTCTCTGTGTTAAATGGAG	<i>Sac I</i> <i>Sal I</i> <i>Sac I</i> <i>Sal I</i> <i>EcoR I</i> <i>BamH I</i>
EMSA	<i>HpWRKY3-C-pGEX-F:</i> GGTTCCGGTGGATCATGCCAGCTGATGATGGCTATAATTGGAGG <i>HpWRKY3-C-pGEX-R:</i> AGTCACGATGCCGGCTGTATTCTCTGTGTTAAATGGAG <i>HpINV2 PRO-Probe-F:</i> ACAAGTCAACCCCTAAATTAAATTGGGGCAATTGGTCAATTG <i>HpINV2 PRO-Probe-R:</i> CAAATGACCAATTGCCCAATTAAATTGGGGCAATTGGTCAATTG <i>HpINV2 PRO-Probe-Mutant-F:</i> ACAAAAAAAACCTAAAATTAAATTGGGGCAATTGGTCAATTG <i>HpINV2 PRO-Probe-Mutant-R:</i> CAAATGACCAATTGCCCAATTAAATTAGGGTTTTTGT <i>HpSuSy1 PRO-Probe-F:</i> GCACCTGTATTGATAATTGACCGTTAATTGAAATT <i>HpSuSy1 PRO-Probe-R:</i> AAAATTCAAATTGGCACTAACACGGTCAATTATCATAAACACAGTGC <i>HpSuSy1 PR-Probe-Mutant-F:</i> GCACCTGTATTGATAAAAAAAACGTTAAAAAAAGCCAATTGGAAATT <i>HpSuSy1 PRO-Probe-Mutant-R:</i> AAAATTCAAATTGGCTTTTAAACGTTTTTATCATAAACACAGTGC	<i>BamH I</i> <i>Not I</i>
Subcellular localization	<i>HpWRKY3-pEAQ GFP-F:</i> CAAATTGCGACCGGTATGCCGAAAACGATGCCGT <i>HpWRKY3-pEAQ GFP-R:</i> TGCTAGTCATACCGGTTATTCTCTGTGTTAAAT	<i>Age I</i> <i>Age I</i>
Trans-activation Activity Assay	<i>HpWRKY3-pGBK7-F:</i> GCCATGGAGGCCAATTGATGCCGAAAACGATGCCGT <i>HpWRKY3-pGBK7-R:</i> GGCGCGTCAGGTGACGCTATGTTATTCTCTGTGTTAAATGGAG <i>HpWRKY3-pBD-F:</i> TCGCCGACCGGTAGGCCATTGCGGAAAACGATGCCGTG <i>HpWRKY3-pBD-R:</i> AACCAAGAGTAAAGGCCCTATGTTATTCTCTGTGTTAAATGGAG	<i>EcoR I</i> <i>Sal I</i> <i>Stu I</i> <i>Stu I</i>
Transient expression	<i>HpINV2 PRO-LUCReporter-F:</i> TATAGGGCGAATTGGGTACCGTTGGAGTGGAGAGGG <i>HpINV2 PRO-LUCReporter-R:</i> TTGGCGTCTCCATGGTAGGAAGAGAAGAGTTTGT <i>HpSuSy1 PRO-LUCReporter-F:</i> TATAGGGCGAATTGGGTACCATATAACTTGTCTGAGAAGGAAAAGGG <i>HpSuSy1 PRO-LUCReporter-R:</i> TTGGCGTCTCCATGGTAGGAAGATGGACGGTCTAGAGAAC <i>HpWRKY3-pEAQ-F:</i> CAAATTGCGACCGGTATGCCGAAAACGATGCCGT <i>HpWRKY3-pEAQ-R:</i> AGTAAAGGCCCTGAGCTATGTTATTCTCTGTGTTAAATGGAG	<i>Kpn I</i> <i>Nco I</i> <i>Kpn I</i> <i>Nco I</i> <i>Age I</i> <i>Xho I</i>

Text 1. Nucleotide sequences of *HpINV2* and *HpSuSy1* promoters. W-box motif were underlined. The probe nucleotide sequence used in EMSA assay is indicated in bold. Translation start site (ATG) was shown in yellow.

>*HpINV2*

AGTTGTTGGAGTGGAGAGGGTCTTGAGGTGAAAGAGAGAGAGCATTGTTGAGAAAGA
ATGAGTCCAAACAGTAAACCTCAAAGTAGGCAATTCTACTTAAATATTCTCCTCTGCTCAAC
GAATTT**ACAAGGTCAAACCTAAAATTTAATTGGGGGCAATTGGTCATTGCCTTCTCC**
ATAAATTTCCCACTTACTTTCTCCAAAAGCAACACCAAAATGAATTAGTGCATACCTGTT
GATGACTCACTTACCAAGTAAATTCTAAATTAAAGGGCTGTTCTATTCGCGTTAGATAGAT
AGATAGATATAAGGAGATGTAAGGAACTTAAGAAGCTGTAAGGAGATGTAAGGAGATGTAAG
GAGCTGTAAGGAAATGTAAGAAGATGTAAGGAGCTGTAAGGATAAAATATAAGGAGTGTGAA
TTGTAGATGAGTTGATAGTATAATTATTACAATGAACGTTGGATGAAAAATAATAATATG
AAAATAATATACTTATTGAAAATAATTAAAGTTAACACAAACAATAAAATATAATAA
TCCCCATAAATTGGTGAATAAGATTCTGCACTTGAGGTGATTTTATGAAACATTAGTTG
ATCGAATAAGCAAAGTATAATAAAATT**TGACTAAATCGAACACTCTTAAATA**
AATT CCTATTTCAGTAAATAATAATAAAATGTATGCTCATCTATAATAATATATTATG
TCGAAACTCAAATATAATTACGGCGTTATTATGTTTATGTATATGTAATGTCTGTCATA
AGATATTATAAATTGTATATTGGTAGTATCATTAAAAAATACATATTGTAACAAAAATA
ATAATGCTATAGAAAATCTATAAGATGTAAGGAGATGTAAGAAAATATAATAAAATA
CTGAAAAAAATGTAACACTCTAACATTCTAACCTGCAAAATAGAACCGGAGCTAGA
CACTTTCTTAAAAAAAGAGAGAGAACTTTGTACACACTCGTGCATCTACCAGCCAAAT
CCAGAGTTGCCACCTCCCCTAACCCCCAAGCCGAGCACCCCCCCCCCGCGCTCTATTAA
ACCCATTCTTCTCCCTCCCTCCCTCCATTCTAACACTCAACCTCAACC
TCAATCAACACAAAATCTCTCTTCA**ATG**

>*HpSuSy1*

ATATAACTTGTCTGAGAAGGAAAAGGGTACGCATTGTTAGAACTAGTTATGATCGATGATGTG
GACGCCCTAAAACATAACTAATGATAAATAGAAAACATCAG**TGACT**ACAAATGCAAT
TGTTGTGATGTTGAAATATTAAAGGTATAAAA**GCACGTGTTATGATAATTGACCCGTTA**
TGACTGCCAAAATTGAAATTGGTTATTGGATGAGAATAATTGAGCTTAAAAATAGATT
TCAGTGTTCGAACCTTGCGGATATTATTTCAGTGGCCAATATCATTAGATCGTATTGTTT
AGGCAATTGCAAATGATATTACCGTAAAGCAACACAAAGATGAAATTAAAGTTACCTATAC
TCATTGAAAGTTAAATTCTTAAAAAAATACAATCTAAAATGATATTAGACTATTGAAAA
AATAAATACCGCAAAAGTTCAAGATGCTAAAGTTATTAAATTAGATTACTCCGTTCAA
TACCTCAAAGTTAGATTCTGCACATCCGATTCTAACATTAAACCTACTATAATTGTTT
ATATCTCGCGATCATAATTATAATAAAAGCATCTTCATAAAATAATTATTCTAACATA
CCTCTAACGACACTTTCTGCCATTGAGATGCGATGTACAAATAGTTCTCCCTCAAAT
CAAAGGCAAGAAAAGCAAACCGTCCCCCCCCCAACCACAAAAAAATAAAATGGGGAC
AAAAGTACAAAACCATGAAATTACGGCGACAAATGTACCCATCAAATCAAACATTGAAAAAGA
TAGCCTTGAATCTAATTAGCAAATGGAAAATGCGTACTATGACACTAACATTCTTGAC
GTATCAGACTTGCAAATCAAATTCTCCCTACTAACAAAGGAAGAGAATTGATAACTAAAGCCA
AACTGTTATTAAAGTTGAAAATTGATATTAAATTGATTATAATTAAATTAAATTACT

TTTAAGCATCATTACTTATATTTTATAATTAGTCTCAAATTACAAAAGTATATTATTATTG
TCATTTTGTCCCCCTTAAACAAAAACTCTAAAATCATCTTCTGTTATTAGATAATTATC
AATAATGTTGGGTACTAAAAAGAGAGGTTAATTAAACATGAATAGTAGTAAGAA
TATTTTATAATTCTAAAGCTAAATTATAAAAAATTCTAAATAAAAGTATTTAATAATAAAATTAA
AAATATTAAAATCTAAATGTATCAAATTGTGGTAACTAAAGTTAATAGTTATGCCA
AAACCCACACGACGCTTTCAATGTGCTCATATTCACTGTGGTTCCCAGCGGCAAGGCCCCC
CATTGGTGCCACTTGTCTGGTGGTATTACCACTCACCTTCGCAGTTCCAATTCTC
TCTCCTTATATACCCCTCTTATTGATTGTTCTCGCTGGCTACTACTTCTCTCCCCC
GACCTCGTTCTAGAACCGTCCATCTCCA**ATG**

Text 2. Sequence of HpWRKY3.

>*HpWRKY3*

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ATGCCGGAAAACGATGCCGTGACTTCCACGAAACCGCCGCCGCCGGATGGCGGTGGG  
GCAGCTGCCACCGACTATTACACTGCCGCCGCGTGGTGGGTCGACTCCATTCTCTGGG  
TCCGGGTTCGGAGCTAGCCCAGGCCGATGACCCCTGGTCTCCAGCTTCTCTGAGAATGAC  
CCGGATTCTGATTGTCGTTCTCTCAGCTTAGCTGGGCCATGGCGTCTCCCCGACCGG  
CTTCCGAGAACAGCTCTAGTGAACCGGACCCTCCGGTCAGGCAGAGCAGGCCGGCGT  
CTGGTGGTTCTCAGCAGCAAGGGTTTCACCATTCCGCCGGCTAGTCCTGCTAGCTGC  
TCGATTCTCCTGGTTATTCCCTGGTCAGGGGCCATTGGATGTCACCAGCAAGCGCTAGC  
TCAAGTTACGGCTCAAGCTGTACAAGCACAGCTAACCTGCATGTGCAAGGCAATTTCATCT  
TCCCTCGGTATAAGGTCTCCGGATCATCTCATTCCAGCCTTATCTGAGTCAATGCCACA  
AAATGTGACAAGTTCTGCCCTAGATTCTACCGAGATAAAAGAACCGGCACAGTACTCTGGC  
GGACCAAAGATCACAGCCTCTTCCATGATTGATAAGCCAGCTGATGATGGCTATAATTGG  
AGGAAATATGGGCAGAACAGTTAAAGGGAGTGAATTCCCCGAAGTTATTACAAATGTACA  
CACATTAATTGCCCTGTCAAGAACAGGTGGAGCGTTCTCTGATGCCAAGTGACCGAGATA  
ATCTATAAGGCCAGCACAAATCATCCGCCACCACAAAAACGTTCTAAAGAGAGCGGAAATTCT  
AATGGAAACTTGGGAAGTCAAGAACCCCTGAATTAAACATGAATAGCACGAATGAGGGCATG  
ACTTACTCATTGTCTAGAACGGATCAGGAATCTAGCCAAGCTACAGCAGAGCATTGTCTAGTG  
ATGAGGAGGAAGTGGCAATGATGAAACTAGAGAATCTGAGGATGAACTGATGCCAAGAGA  
AGGAACACAGAACTCCGGTACCAAGAGCAAGCTGTTCACATAGGACAGTCACTGAACCAA  
GATTATTGTTCAAACAACTAGTGAAGTTGATCTTGGACGATGGTATAGGTGGCGCAAGTAT  
GGACAAAAAGTTGTCAGAACATTCAATCCGAGGAGCTATTACAAATGCACTACCCAGGG  
TGTAATGTCGTAAGCATATTGAGCGGGCAGCCTCAGACCCCTAAAGCAGTGATAACAAACATACG  
AGGGGAAGCACAGTCACGATGTTCCAGCTGCTAACAGACGAGCAGCCATAACACATCCAACAGC  
AGCTTTCACAGCCAAGACCGCTAGTTGGCAGCGAATAATAACTCCTATGTTGGGAAGACG  
GATTTACAAGTAATCAACAGCCTGTAGCCCTCTCCATTAAAACAAGAGAGAATAACATAG
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