

A WD40 Repeat Protein from *Camellia sinensis* Regulates Anthocyanin and Proanthocyanidin Accumulation Through the Formation of MYB–bHLH–WD40 Ternary Complexes

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TableS 1. The specific primers used in this study.

| purpose | Gene | | primer sequence (5'-3') |
|--|--------------------|--------------------------------------|--|
| | names | primer name | |
| cloning | <i>CsWD40</i> | <i>CsWD40</i> -attB-HA-F | GGGGACAAGTTTGTACAAAAAAGCAGGCTATGTACCCATACGATGT TCCAGATTACGCTATGGAGAATTCGAGCCAAG |
| | | <i>CsWD40</i> -attB-HA-R | GGGGACCACTTTGTACAAGAAAGCTGGGTTCAACTTTCAGAAGC TGC |
| | | End-to-end F | ATGGAGAATTCGAGCCAAG |
| | | End-to-end R | GATCTCAAACCTTCAGAAGCTGC |
| Real time PCR | <i>CsWD40</i> | qRT-PCR-F | AAACTCCGTGACCTACGACTCCCCG |
| | | qRT-PCR-R | CTTGGTGGGAGGGTAAGGGTGTTCG |
| Construction of protein expression vectors | <i>CsWD40</i> | <i>CsWD40</i> -AD-F | ATGGAGGCCAGTGAATTCATGGAGAATTCGAGCCAAG |
| | | <i>CsWD40</i> -AD-R | CTCGAGCTCGATGGATCCTCAAACCTTCAGAAGCTGC |
| | | <i>CsWD40</i> -BD-F | GCCATGGAGGCCGAATTCATGGAGAATTCGAGCCAAG |
| | | <i>CsWD40</i> -BD-R | CTGCAGGTCGACGGATCCTCAAACCTTCAGAAGCTGC |
| | <i>CsMYB5a</i> | <i>CsMYB5a</i> -AD-F | ATGGAGGCCAGTGAATTCATGGGAGGAGTCCATGCTG |
| | | <i>CsMYB5a</i> -AD-R | CTCGAGCTCGATGGATCCTCATGGCCAGTCCTCAGAAT |
| | | <i>CsMYB5a</i> -BD-F | GCCATGGAGGCCGAATTCATGGGAGGAGTCCATGCTG |
| | | <i>CsMYB5a</i> -BD-R | CTGCAGGTCGACGGATCCTCATGGCCAGTCCTCAGAAT |
| | <i>CsMYB5e</i> | <i>CsMYB5e</i> -AD-F | ATGGAGGCCAGTGAATTCATGGGAAGGGCTCCTTGTTG |
| | | <i>CsMYB5e</i> -AD-R | CTCGAGCTCGATGGATCCTCAGATCAACAAAGATTCAG |
| | | <i>CsMYB5e</i> -BD-F | GCCATGGAGGCCGAATTCATGGGAAGGGCTCCTTGTTG |
| | | <i>CsMYB5e</i> -BD-R | CTGCAGGTCGACGGATCCTCAGATCAACAAAGATTCAG |
| | <i>CsAN2</i> | <i>CsAN2</i> -AD-F | ATGGAGGCCAGTGAATTCATGGACATTGTTTGTGTGTTT |
| | | <i>CsAN2</i> -AD-R | CTGCAGGTCGACGGATCCTCATTATCACCTAACAGATCC |
| | | <i>CsAN2</i> -BD-F | GCCATGGAGGCCGAATTCATGGACATTGTTTGTGTGTTT |
| | | <i>CsAN2</i> -BD-R | CTGCAGGTCGACGGATCCTCATTATCACCTAACAGATCC |
| <i>CsTT8</i> | <i>CsTT8</i> -AD-F | ATGGAGGCCAGTGAATTCCTCTGGTGTGGATTACC | |
| | <i>CsTT8</i> -AD-R | CTCGAGCTCGATGGATCCTCAGTTCTGGGGTATTAT | |
| | <i>CsTT8</i> -BD-F | GCCATGGAGGCCGAATTCCTCTGGTGTGGATTACC | |

| | | | |
|------------------------|---------------|-----------------------|--------------------------------------|
| | | <i>Cs TT8</i> -BD-R | CTGCAGGTCGACGGATCCTCAGTTCTGGGGTATTAT |
| | | <i>CsGL3</i> -AD-F | ATGGAGGCCAGTGAATTCATGTGTTGGGCAATGGCT |
| | <i>CsGL3</i> | <i>CsGL3</i> -AD-R | CTCGAGCTCGATGGATCCCTAACACTTGCCAGCAAT |
| | | <i>CsGL3</i> -BD-F | GCCATGGAGGCCGAATTCATGTGTTGGGCAATGGCT |
| | | <i>CsGL3</i> -BD-R | CTGCAGGTCGACGGATCCCTAACACTTGCCAGCAAT |
| | <i>NtCHS</i> | <i>NtCHS</i> -qRT- F | TGCTAAGCGAATACGGGAAC |
| | | <i>NtCHS</i> -qRT- R | AACAGAAACACTGCGGAGGA |
| | <i>NtF3'H</i> | <i>NtF3'H</i> -qRT- F | TTGTCCCGAATGACTTACG |
| | | <i>NtF3'H</i> -qRT- R | GCATAGTAGGTAGCGGAGGTG |
| | <i>NtDFR</i> | <i>NtDFR</i> -qRT- F | GCGAAAGGGAGGTATATGTGCTC |
| | | <i>NtDFR</i> -qRT- R | TGCTTGTCCCTCGGTACTCAGTA |
| | <i>NtFLS</i> | <i>NtFLS</i> -qRT- F | TGAAGGGAAAAGGGGTTGG |
| | | <i>NtFLS</i> -qRT- R | ACTCCTCATTGCTTCCCTGTAG |
| | <i>NtLAR</i> | <i>NtLAR</i> -qRT- F | GCAGCAGAAGACTATAGAAGTGTG |
| | | <i>NtLAR</i> -qRT- R | CATGTGTTAGAGCTGCAACTACAC |
| Quantitative RT-PCR | <i>NtANS</i> | <i>NtANS</i> -qRT- F | ACTACTACCCCAAATGTCCCAAC |
| | | <i>NtANS</i> -qRT- R | CCGTTACCCACTGTCTTCATAGA |
| | <i>NtANR</i> | <i>NtANR</i> -qRT- F | CTTGAAGGTATGCAGATGTT |
| | | <i>NtANR</i> -qRT- R | GCAGAGCAAACATATCGTCCAG |
| | <i>NtAN2</i> | <i>NtAN2</i> -qRT- F | GAAGAAAGGTGCATGGACTG |
| | | <i>NtAN2</i> -qRT- R | TCTGCAGCTCTTCTGCATC |
| | <i>NtAN1a</i> | <i>NtAN1a</i> -qRT- F | ACCATTCTCGAACCCGAAG |
| | | <i>NtAN1a</i> -qRT- R | TGCTAGGGCACAATGTGAAG |
| | <i>NtAN1b</i> | <i>NtAN1b</i> -qRT- F | CTTGAACACTTCTCA AACCGA |
| | | <i>NtAN1b</i> -qRT- R | TGCTAGGGCACAATGTGAAG |

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CSWD40  MENSQDS.HLRSE.....NSVYDSYFLYAMAFSSARSFP.HHHRVAGSEHEVNRVDIVSEF...EETLMTKNG...EGRYPPTK  83
PHAN11  MENSQDSQHLRSE.....NSVYDSYFLYAMAFSSFFTP...RRRVAAGSEHELNRRVVELLSFN...EETLLNPH...EGRYPPTK  81
GHITG1  MENSQDS.HLRSD.....NSVYDSYFLYAMAFSSSTPSTN.INHQVAGSEHELDVNRVDIISFD...PETLSFRTH...EGRYPPTK  84
ATTG1   MNSAPDS.LRSE.....TAVYDSYFLYAMAFSSLRSS...SGHRVAGSEHELDVNRVDIISFD...SDSMTVKPL...EGRYPPTK  81
INWD    MVNSTQGS.NLRSE.....NSVYDSYFLYAMAFSSFAAHHGLRRRSVAVGSEHEVNRVVELLSEF...EDTVLKRTH...EGRYPPTK  85
PFWD    MENSQDS.HRPE.....NVVYDSYFLYAMAFS.AVN...RRRVAAGSEHELNRRVVELLSFN...EDSSSLRFT...EGRYPPTK  78
VWVDR1  MERSSQES.HLRSE.....NCVYDSYFLYAMAFSSSTQH...HHPRVAGSEHELDVNRVDIISFD...EDALAIRTH...EGRYPPTK  81
MTWD    MNSQDSQSHLRSDN.....NSVYDSYFLYAMAFSSFN.TNSP...CQRVAGSEHELDVNRVDIISFD...PDTLSLRFT...EGRYPPTK  82
ZMPAC1  MDPPKPSVAVSSGPEPFPNPHATCELFHSIYALAFSPVAP.....VLAGSEHELDVNRVDIISFDVPRPSAASFRAI...EGRYPPTK  88
VWVDR2  MGASDPYQDASDE...CQKRSEIYVEAFWHIYAMNWSVRDK...KYRVAAGSEHELDVNRVVEVLQD...DSTGEIRSD...EGRYPPTK  86
MDITG1  MENSQDS.HLRAE.....NSVYDSYFLYAMAFSPQTRTR.HQHRRVAGSEHEVNRVDIISFD...PDTLSIRKPH...EGRYPPTK  84
PITG1   MENSAPDS.HLQSE.....NSVYDSYFLYAMAFSPQTRNR.HQHRRVAGSEHEVNRVDIISFD...PDTLSIRKPH...EGRYPPTK  84
MITG1   LIFSEPSLR.....AAVYDSYFLYAMAFSSSR...AGQVAGSEHELDVNRVDIISFD...SDSMTVKPL...EGRYPPTK  69
FGWD40  MNSQDSQSHLRSD.....HSVYDSYFLYAMAFSPRRHGH...NHRVAGSEHEVNRVDIVSEF...PDTLLKRF...EGRYPPTK  83
Consensus  t          a s e n          p l f h y p p t k

CSWD40  LMFENFAS...LRKSSDILASSGDYFLRW...EVRD..NSIEISILNNSKTS  EFCALTSFDWNEVDARRIGTSIDTTCTWD  ERGWVE  169
PHAN11  LMFENFIK...SNN..DILASSGDYFLRW...EVKE..SSIEPLTILNNSKTS  EYCALTSFDWNEVEPRIGTSIDTTCTWD  EKGWVE  164
GHITG1  LMFENRKSASSSSCSDLASTGDFLRW...EVRE..SSIEPVTILNNSKTS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  EKGWVE  172
ATTG1   LMFSEPSLR...RPSGDLASSGDYFLRW...EINEDSSTVEPISVLNNSKTS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  EKGWVE  168
INWD    LMFENFTA...SMKSADLVSSGDYFLRW...EVRE..ASIEPVSILNNSKTS  EYCALTSFDWNEVEPRIGTSIDTTCTWD  EKGWVE  170
PFWD    LMFSEVSA...FSN..LASSGDYFLRW...EVRD..SSIVAVSILNNSKTS  EYCALTSFDWNEVEPRIGTSIDTTCTWD  EKGWVE  160
VWVDR1  LMFENFS.....RTSPDFHASSGDYFLRW...EVRD..NSIQPLSVLNNSKTS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  ERGWVE  163
MTWD    LMFENFATHSSLQRTSSDLASTGDFLRW...EVRE..NSVEALSILNNSKTS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  ERGWVE  169
ZMPAC1  LMFENR.....AAAFSLIASSADLRIWHPTLDDLSDTAPAPELRSVLDNRKAS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  DRGWVE  177
VWVDR2  LMFENFKD...CQRFDLASTGDFLRW...NISD..DRVELKOLLNNSKTS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  ERRTVD  169
MDITG1  LMFENFNA...LHKINDVASSGDYFLRW...EVGD..SIVEPIQLNNSKTS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  EKGWVE  169
PITG1   LMFENFNS...LHKSNDVASSGDYFLRW...EVRD..SSVEPIQLNNSKTS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  EKGWVE  169
MITG1   LMFSEPSLR...RPSGDLASSGDYFLRW...EVSEDSSTVEPISVLNNSKTS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  EKGWVE  156
FGWD40  LMFSEPSLR.....SDLASSGDYFLRW...EVRD..SSVEPVSILNNSKTS  EFCALTSFDWNEVEPRIGTSIDTTCTWD  EKSQVE  161
Consensus  f p          l          lr          s e p l t s f d w n e v e p r i g t s i d t t c t w d          v

CSWD40  TQLAHDREYNDIAWCEAGVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  265
PHAN11  TQLAHDREYNDIAWCEAGVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  260
GHITG1  TQLAHDREYNDIAWCEARVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  268
ATTG1   TQLAHDREYNDIAWCEARVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  264
INWD    TQLAHDREYNDIAWCEAGVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  266
PFWD    TQLAHDREYNDIAWCEAGVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  256
VWVDR1  TQLAHDREYNDIAWCEAGVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  259
MTWD    TQLAHDREYNDIAWCEARVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  265
ZMPAC1  TQLAHDREYNDIAWCEAGVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  273
VWVDR2  TQLAHDREYNDIAWCEAGVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  265
MDITG1  TQLAHDREYNDIAWCEARVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  265
PITG1   TQLAHDREYNDIAWCEARVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  265
MITG1   TQLAHDREYNDIAWCEARVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  252
FGWD40  TQLAHDREYNDIAWCEARVFSVSADGSVRFILRDKEHSTIYESIPDTEFLRIANNQDILRYWATIIMPENKRWLILRSSTIPVAELRRR  257
Consensus  tqla h d r e y n d i a w c e a g v f s v s a d g s v r f i l r d k e h s t i y e s i p d t e f l r i a n n q d i l r y w a t i i m p e n k r w l i l r s s t i p v a e l r r r

CSWD40  ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEMSYVAGAEIQLQWAAALPFWIAVPSAAQIQK  341
PHAN11  ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEMSYVAGAEIQLQWSPAQRWIAVPSAAQIQK  336
GHITG1  ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDECVYVAGAEIQLQWSPAQRWIAVPSAAQIQK  344
ATTG1   ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEMSYVAGAEIQLQWSSSQPFWIAVPSAAQIQK  340
INWD    ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEMSYVAGAEIQLQWAAQPFWIAVPSAAQIQK  342
PFWD    ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEITVYVAGAEIQLQWAAQPFWIAVPSAAQIQK  332
VWVDR1  ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEMSYVAGAEIQLQWAAQPFWIAVPSAAQIQK  335
MTWD    ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEITVYVAGAEIQLQWAAQPFWIAVPSAAQIQK  341
ZMPAC1  ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEVLVWVAGAEIQLQWAAQPFWIAVPSAAQIQK  352
VWVDR2  ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDELLVWVAGAEIQLQWSSSQPFWIAVPSAAQIQK  343
MDITG1  ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEMSYVAGAEIQLQWAAQPFWIAVPSAAQIQK  341
PITG1   ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEMSYVAGAEIQLQWAAQPFWIAVPSAAQIQK  341
MITG1   ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEMSYVAGAEIQLQWSSSQPFWIAVPSAAQIQK  328
FGWD40  ASVNAIWAQCSHRFICSGEDDSCALIMVPTVAGPN...GDEMSYVAGAEIQLQWAAQPFWIAVPSAAQIQK  333
Consensus  a s v n a i w a q c s h r f i c s g e d d s c a l i m v p t v a g p n . . . g d e m s y v a g a e i q l q w a a l p f w i a v p s a a q i q k

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Figure S1. Amino acid alignment of CsWD40 proteins from tea plants and its orthologs from other plants

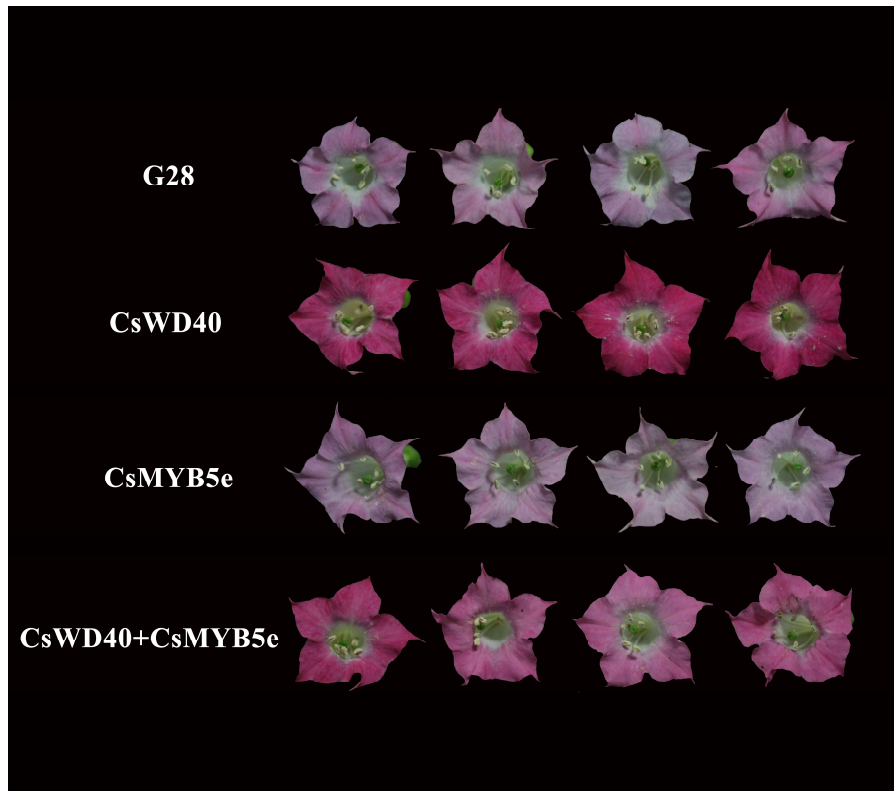


Figure S2. Flower pigmentations of *CsWD40*, *CsMYB5e*, and *CsWD40*♀*CsMYB5e*♂ (or *CsWD40*♂*CsMYB5e*♀) transgenic tobaccos.