

Figure S1. Carotenoid (A) and capsaicinoid (B) biosynthetic pathways. A. In the first step, Geranylgeranyl pyrophosphate (GGPP) is catalyzed by a series of synthetases, and transformed to lycopene. Then, there are two different branches: α -carotene finally is developed into lutein, and β -carotene is catalyzed to transform into capsorubin or capsanthin by capsanthin/capsorubin synthase (CCS). The ellipsis indicates intermediate steps. B. Phenylalanine and chain fatty acid biosynthesis pathways which are adjusted by a series of genes encoding enzymes synthesize capsaicin together.

ZDS, ζ -carotene desaturase; CRTISO, carotenoids isomerase; LCYE, lycopene ϵ -cyclase; CrtZ-2, β -carotene hydroxylase-2; PAL, phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumaroyl-CoA ligase; HCT, hydroxycinnamoyl transferase; C3H, p-coumaroyl shikimate/quinate 3-hydroxylase; COMT, caffeoyl-CoA 3-O-methyltransferase; HCHL, hydroxycinnamoyl-CoA hydratase lyase; AMT, aminotransferase; BCAT, branched-chain amino acid aminotransferase; Kas, ketoacyl-ACP synthase; ACL, acyl carrier protein; and FatA, acyl-ACP thioesterase.

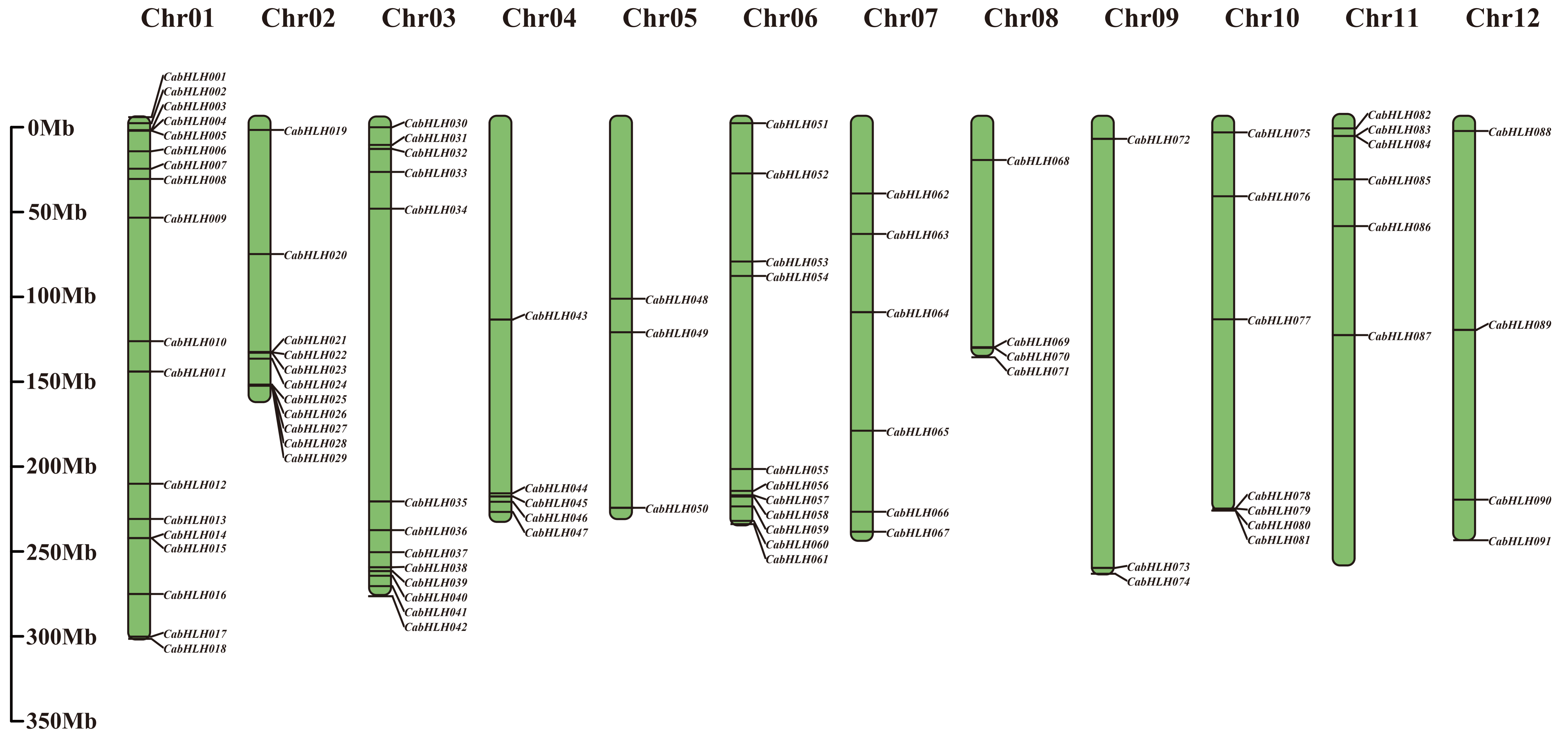


Figure S2. Chromosomal localizations of *CabHLHs*. The chromosome distribution of each *CabHLH* was obtained from the 2.0 version *Capsicum annuum* genome annotation information, and TBtools (1.068 version) were used to make the linkage visualization.

	basic	helix 1	loop	helix 2	
CabHLH001	I AKNHVEAERLRREKLNHRFYALRS	..	VVPNVK	MDKASLLADAVTY	I NELKAKVEE
CabHLH061	EALNHVEAERORREKLNQRFYALRA	..	VVPNI K	MDKASLLGDAL AY	I TDLQARI RV
CabHLH097	ATDSHSLAERVRRREKISERMRFALQD	..	LVPGCKI	TGKAGMLDEI I NY	VQSLOROVF
CabHLH006	EPLNHVEAERORREKLNQRFYALRA	..	VVPNVK	MDKASLLGDAL SY	I NELKSKLHN
CabHLH082	.. KSHSEAERRRRERI NNHLAKLRS	..	LLPNTK	TDKASLLAEVI QH	VKELKRQTS L
CabHLH066	.. KNHSEAERRRRERI NGHLGTLRN	..	LI PGTK	MDKAALLAKVI GH	I KELRMNAE
CabHLH034	.. RSHSEAERRRRDRINAQLS TLRK	..	LI PTKS	MDKAALLGS VI DH	VKDLKGRSTE
CabHLH037	ATDSHSLAERVRRREKISERMKMLQS	..	LVPGCKV	TGKALMLDEI I NY	VQSLOROVF
CabHLH023	.. KSHSEAERRRRQRI NAHLS TLRN	..	LVPSSK	MDKAALLAQVVCQ	VKQLKETATH
CabHLH004	.. SKNI VSERNRRRKLNERLFAIRA	..	VVPNI K	DKAS I KDAI DH	I QDLHEQERR
CabHLH005	.. SKNI VSERNRRRKLNERLFAIRA	..	VVPNI K	DKAS I KDAI DH	I QDLHEQERR
CabHLH017	KMDI HVEAERKRREKLNHRFYALRS	..	VVPYVK	MDKASLLADAVTY	I NELKANI ED
CabHLH102	.. I NHKEAERRRRORI NS HLHTLRT	..	LLSCNK	TDKASLLAKVVQR	VRELKEQTS K
CabHLH092	GHELHI RTERERRRKRMNMFEELOT	..	LLP.. HQ	DKSSI VDETVR	.. LS TVN
CabHLH078	.. LNHNAS ERDRRKKI NGLYS S LRS	..	LLPASHK	LSI PATVS KI LKY	I PELQNEVER
CabHLH079	.. LNHNAS ERDRRKKI NDLYF S LRS	..	LLPASHK	LSI PSTI SRI LKY	I PELQSEVER
CabHLH052	.. I LHRDI ERHRRHEMATLCATLRS	..	NLSYETK	RSLS DHVQAVNY	I KLENNI KE
CabHLH035	.. I I HRDI ERORRQENS ALYS S LRS	..	LLPLQI K	RSVS DHVHEAVNY	I KEMQANI KE
CabHLH011	ATNSHSLAERVRRREKISERMKFLQD	..	LVPGCKV	TGKAVMLDEI I NY	VQSLOROVF
CabHLH043	NPLQPNLFENASVOPSPDAEHCTS Q	..	LYGPGK	.. QDTI ES DVLS Y	GRNLQEQMKI
CabHLH054	KKVMHRETERORRQENS TLYACLQ	..	QLPLEI R	.. STS DHI LVAANY	I EQLOKQVH
CabHLH008	KMSAQSI AARORRKKI TEKTQELGK	..	LI PGGK	MNTAEMLQATFEY	I KFLQAQAGL
CabHLH059	ATDSHSLAERVRRREKISERMKI LQA	..	LVPGCKV	TGKALMLDEI I NY	VRS LONQVEF
CabHLH015	.. DMKHHAS EKLRRRI HFS DKFQALRT	..	LI PNPKN	DRATI I VDAI GY	I NELKKEMMR
CabHLH027	S DQHHI I AERKRREKLS QRFVLSA	..	I LPDLK	VDKASI LEQAI NH	VKELQEKVQ
CabHLH016	.. DSI AI QS GRAEACELMQLDMS E	..	AI RLGS	PDGSDNMS DLNLV	GAGLNTTES F
CabHLH046	ATNSHSLAERVRRERI SERMRLQE	..	LVPGCKI	TGKAVMLDEI I NY	VQSLQOOVEF
CabHLH022	S KDPQSVAARHRRERI SERI RI LQR	..	LVPGCK	MDTASMLDEAVHY	VKFLKKQLQS
CabHLH009	ATHPRS I AERMRRTRI SERMKQLQD	..	LFPNMK	TNTADMLDLAVDY	I KDLQKQVOT
CabHLH072	ATDAHSLAERVRRREKISERMKLLQD	..	LVPGCKV	TGKAVMLDEI I NY	VQSLOROVF
CabHLH051	AAEVHNS ERKRRDRI NEKMRVLQE	..	LI PRCK	ADKVS MLDEAI EY	LKSLQLQVQM
CabHLH044	SAYTHAVAERKRREERI NGHLHTLKK	..	LFPYLK	KDKPRVLTEAVTQ	LKEI AQQLEL
CabHLH010	LSQEH I LAERKRREFS HLFVAVLAK	..	TI PGLK	LDKAS I LEDAI KY	I GELQERVST
CabHLH024	KTADRKTI EKNRRS QMKDLYMKLNS	..	LVHHDHF	SSLPDQLEEAANY	I KKLQI DLEK
CabHLH075	DNSKELI HGPS YNNDVNS NLHLVDD	..	I VESHEF	FRATTI CDNS KEL	VHGLHLVDDI
CabHLH013	TPSDSI QKERKRRGKMAEVYSLQS	..	LVPSI HA	TREKI VTESTDY	I KRLEEEI LR
CabHLH105	GHELHI RTERERRRKRMNMFEELOT	..	LLP.. HQ	DKSSI VDETVRY	I KMLENTVDN
CabHLH094	GHELHI RTERERRRKRMNMFEELOT	..	LLP.. HQ	DKSSI VDETVRY	I KMLENTVDN
CabHLH104	GHELHI RTERERRRKRMNMFEELOT	..	LLP.. HQ	DKSSI VDETVRY	I KMLENTVDN
CabHLH101	GYEI HI WTERERRRKRMNMFENLOT	..	LLP.. HQ	DKSSI VDEAVRY	I KILQTTVTN
CabHLH014	.. TKHFATERORRVHLNDKYKALRS	..	VVPNPKT	DRASI VKDAI DY	I NELKRGVNE
CabHLH033	.. VVHRNVERHRRKEMANLVNS LRS	..	LI PI EI K	RSAS DHVQAVNY	I KHLQKNI QE
CabHLH002	PDKNRV I SERRRREK I NERFMHLS	..	MLPTS K	VDKVSLLEDEI AY	MKELERRVOD
CabHLH021	AAEVHNS EKRRRS RI NEKMKALQK	..	LI PNS K	TDKAS MLDEAI EY	LKQLQLOVQM
CabHLH036	NRI SHI TVERNRRKQVNEHLS VLRS	..	LMP CFA	.. DQASI I GGVVDY	I NELQOVLQS
CabHLH050	QRMTHI AVERNRRKQVNEHLRV LRS	..	LMPGS V	.. DQASI I GGAI EF	VRELEQLLOC
CabHLH030	QRMHI AVERNRRKQVNAHLV LRS	..	LMPSS V	.. DQASI VGGAI EF	VRELEQLLOC
CabHLH012	.. MS HI AVERNRRKQVNEHLKI LRS	..	LTPCFI	.. DQASI I AGVI EF	I KELHLVLOS
CabHLH071	QRI THI AVERNRRKQVNEYLS VLRT	..	LMPGS V	.. DQASI VSAAI NY	VKELEQLOF
CabHLH088	QRMTHI AVERNRRKQVNDYLAI LRS	..	LMPPSA	.. DQASI VGGAI NF	VKELEQLOF
CabHLH068	PQSKNLMAERKRRKLNRLYALRA	..	LVPKI K	DRASI LGDAI EY	VMELEKQVKD
CabHLH077	ATDPQSLYARKRREERI NERLKI LQS	..	LVPNGK	VDI STMLEEAVQY	VKFLQLQI KL
CabHLH007	YQSKNLVTERKRRNRI KDGLFALRA	..	LVPNI K	DKVAI LGDAI DY	I NLOEKVKL
CabHLH086	YQSKNLVTERKRRNRI KDGLFALRA	..	LVPNI K	DKVAI LGDAI DY	I NLOEKVKL
CabHLH106	ATDPQSLYARKRREERI NERLRLTQS	..	LVPNGK	VDI STMLEEAVQY	VKFLQLQI KL
CabHLH053	DRSRTL I SERKRRGRMKEKLYALRS	..	LVPNI K	DKAS I GDAL LY	VOGLQTKAKI
CabHLH090	SSDPQSVAARHRRERI SEKI RI LQR	..	LVPGCK	MDTASMLDEAI HY	MKFLKKQVQS
CabHLH098	STDPQSVAARHRRERI SERI RI LQR	..	LVPGCK	MDTASMLDEAI HY	VKFLKNQVQS
CabHLH003	MASKNI MSERNRRKLNRLFAIRA	..	VVPNI K	DKAS I KDAI EY	I QDLHEQERR
CabHLH064	MASKNI VSERNRRKLNRLFAIRA	..	VVPNI K	DKAS I KDAI DY	I QDLHEQERR
CabHLH085	ATDSHSLAERARREKISERMKVLQD	..	LVPGCKV	I GKALVLDDEI I NY	I QSLQHQVEF
CabHLH041	LPAKNLMAERRRRKLNDRLYMLRS	..	VVPKI K	DRASI LGDAI EY	LRELLQTI ND
CabHLH080	ATDSHSLAERARREKINARMKLLQE	..	LVPGCKI	SGTAVVLDDEI I NH	VQSLOROVF
CabHLH026	QPSKNLMAERRRRKLNDRLS MLRS	..	VVPKI K	DRTS I LGDTI DY	VKELLDKI SK
CabHLH045	QPSKNLMAERRRRKLNDRLS MLRS	..	VVPKI K	DRTS I LGDTI DY	MKELLEKI NK
CabHLH076	.. LNHVEAERORREKLNHRFYALRS	..	VVPHVK	MDKASLLS DAVAY	I NELSKVDE
CabHLH070	MAMNHVEAERORREKLNRFYALRS	..	VVPNVR	MDKASLLS DAVSY	I NQLKARVDE
CabHLH087	LGKRTS EAARLHPPLYEHPLOVLS Q	..	SEAEDE	GEEFYKREDPNNGI	I PSTKELVKT
CabHLH084	EVI QYVQYLOETVQKYEYS YOPMS S	..	LMPWRS	.. VQSLP VOPHALKNGI	GPLVTS TMQ
CabHLH018	EPLNHVEAERORREKLNQRFYALRA	..	VVPNVK	MDKASLLGDAL AY	I NELKSVQON
CabHLH107	.. QDHI I AERKRREKLS QRFI ALSA	..	LI PGLK	MDKAS VLGDAI KY	LKQLQERVKT
CabHLH038	ATDSHSLAERARREKISERMKI LQD	..	LVPGCKV	I GKALVLDDEI I NY	I QSLOROVF
CabHLH049	EVI QYVQYLODKVQKYEYS YOPMS S	..	LMPWRS	.. VQSLP VOPHALKNGI	GPLVTS TMQ
CabHLH073	TQVHHMI SERRRREKLNENFOHLRS	..	LLPLGK	KDKAS VLAS TTDY	I SCLKDQVKE
CabHLH091	ATHPRS I AERVRRTRI SERMKQLQE	..	LVPNMK	TNTADMLDFEADY	I KELETQVKA
CabHLH028	.. QDHVLAERKRRERLTQYFVTLST	..	LI PNLK	LDKATVLDGAI KY	I KOLEEQVKS
CabHLH029	.. QDHVLAERKRRERLTQYFVTLST	..	LI PDLK	LDKAS I LGDAI KY	I KOLEEQVKS
CabHLH103	ATHPRS I AERERRTRI S GKLKLLQD	..	LVPNMK	TSYADMLDLAVQH	I RTLQDQVQV
CabHLH062	ATNSHSLAERI RREKISERMKYLQD	..	LVPGCKV	TGKAVMLDEI I NY	VQSLOROVF
CabHLH048	EPLNHVEAERORREKLNQRFYALRA	..	VVPNI K	MDKASLLGDAL AH	I TDMQKRI RD
CabHLH058	RAAI HNQAERRRRDRINAQKMLQK	..	LVPNAK	TDKASMLDEVI RY	LKQLQAQVQL
CabHLH081	ATHPRS I AERVRRTRI SERMKQLQE	..	LVPNMK	TNTADMLDLAVEY	I KGLQKQYKV
CabHLH040	AAAVHNQSERRRRDRINAQKMLQK	..	LVPNAK	TDKASMLDEVI DY	LKQLQAQVQF
CabHLH032	ATDPHSI AERLRREKISERI KALQE	..	LVPSCK	.. DRAAMLDEI LDY	VKFLRLQVQV
CabHLH060	ATDSHSLAERVRRREKISERMKLLQD	..	LVPGCKV	TGKALMLDEI I NY	VQSLOROVF
CabHLH042	STDPQTVAAARORREERI SERI RVLOR	..	LVPGCK	MDTASMLDEAANY	LKFLRSQVKA
CabHLH063	AAEVHNS ERKRRDRI NEKMKALQD	..	LLPHSK	TDKASMLDEAI EY	LKSLQMLQOM
CabHLH099	AAEVHNS ERKRRDRI NEKMRALQE	..	LI PNCK	ADKASMLDEAI EY	LKTLQLQVQI
CabHLH025	TKDPQSI AAKNRRERI SERLKI LQE	..	LVPNGK	VDLVTMLEKAI GY	VKFLQLQVQV
CabHLH100	SAEVHNS EKRRRS RI NEKMLKALQD	..	LI PNS K	TDKASMLDEAI EY	LKQLQLQVQM
CabHLH039	EVI EYI HFLOGKVHKEYES YQGVNDNEP	..	LKPHRT	THGVS NHPQCTI N	.. LEPNIST
CabHLH057	EVI EYI QFLOEKVHKEYES YQGLNEKP	..	STPNSM	AGLI DHS OGTNS	.. LEPKRTS
CabHLH069	EPLNHVEAERORREKLNQRFYALRA	..	VVPNI K	MDKASLLGDAL AY	I TELQKRLRD
CabHLH067	DAVRMVTQLRDEAQKLDKSNLNLQE	..	KI KELALAVEKTELREDEKOR	..	LKALEQQLKT
CabHLH031	DAI RVLNQLKTES EYKEMNQLVE	..	EI KMLA	.. EKNELRREKLA	LKAMEQELKA
CabHLH095	DAVRVVTQLRGEAQKLDKSNLNLQE	..	KI KELALSVEKHELREDEKOR	..	LKSLQOQLKT
CabHLH096	DTVQMLKDLTAQVSR LKS EYAAALTD	..	ESRELO	.. EKNDLREKTS	LKSLNAQYQO
CabHLH074	ATDPHSI AERLRREKISERMKYLQD	..	LVPNAKT	.. DKASMLDEI I DY	VRFLQLOVQV
CabHLH083	EVI QYVQYLOETVQKYEYS YOPMS S	..	LMPWRS	.. VQSLP VOPHALKNGI	GPLVTS TMQ
CabHLH056	LPAKNLMAERRRRKLNDRLYMLRS	..	VVPRI K	DRASI LGDAI EY	LKELLOKI ND
CabHLH093	TQVHHMI SERRRREKLNENFOHLRS	..	LLPLGK	KDKAS VLAS TTDY	I SCLKDQVKE
CabHLH055	ATHPRS I AERVRRTRI S DRI RKLQE	..	LVPNMK	TNTADMLDEAVEY	VKFLQROI OE
CabHLH089	ERLNHAEVERORRAKLNQQFNALRA	..	VVPNI K	KDKVSLGGAI AH	I TDMQKRI RD
CabHLH047	DRSRTL I SERKRRGRMKEKLYALRS	..	LVPNI K	DKAS I GDAL LY	VOGLQTKAEI
CabHLH065	TQVHHMI SERRRREKLNDS FOLLRS	..	LLPPAK	KDKAS VLAS TTEY	LSSLKQVQVEE
CabHLH019	ATDSHSLAERARREKISERMKYLQD	..	LVPGCKV	TGKAGMLDEI I NY	VQSLQKQVEF
CabHLH020	QPSKNLMAERRRRKLNDRLS MLRS	..	I VPKI K	DRTS I LGDAI DY	MKELLEKI HT

Figure S3. Multiple alignment analysis of the bHLH domain in pepper bHLH proteins. Multiple alignments were performed by Clustal X 2.1 with the default parameters. Amino acids with greater than 75% are shown in pink, those with 50 to 75% identity are shown in cyan, and those with 33 to 50% identity are shown in yellow. Dotted lines indicate gaps.

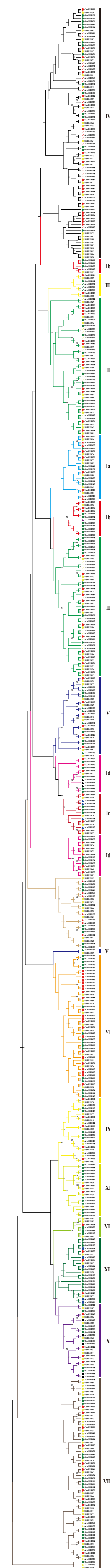


Figure S4. Phylogenetic tree of the bHLH family in *Capsicum annuum*, *Arabidopsis thaliana*, tomato, and rice. The different-coloured branches indicated different groups of bHLH proteins. Red, yellow and green circles represented bHLH proteins in *Capsicum annuum*, tomato and rice, respectively. Other different colours and shapes indicated different groups in phylogeny of *Arabidopsis thaliana*.

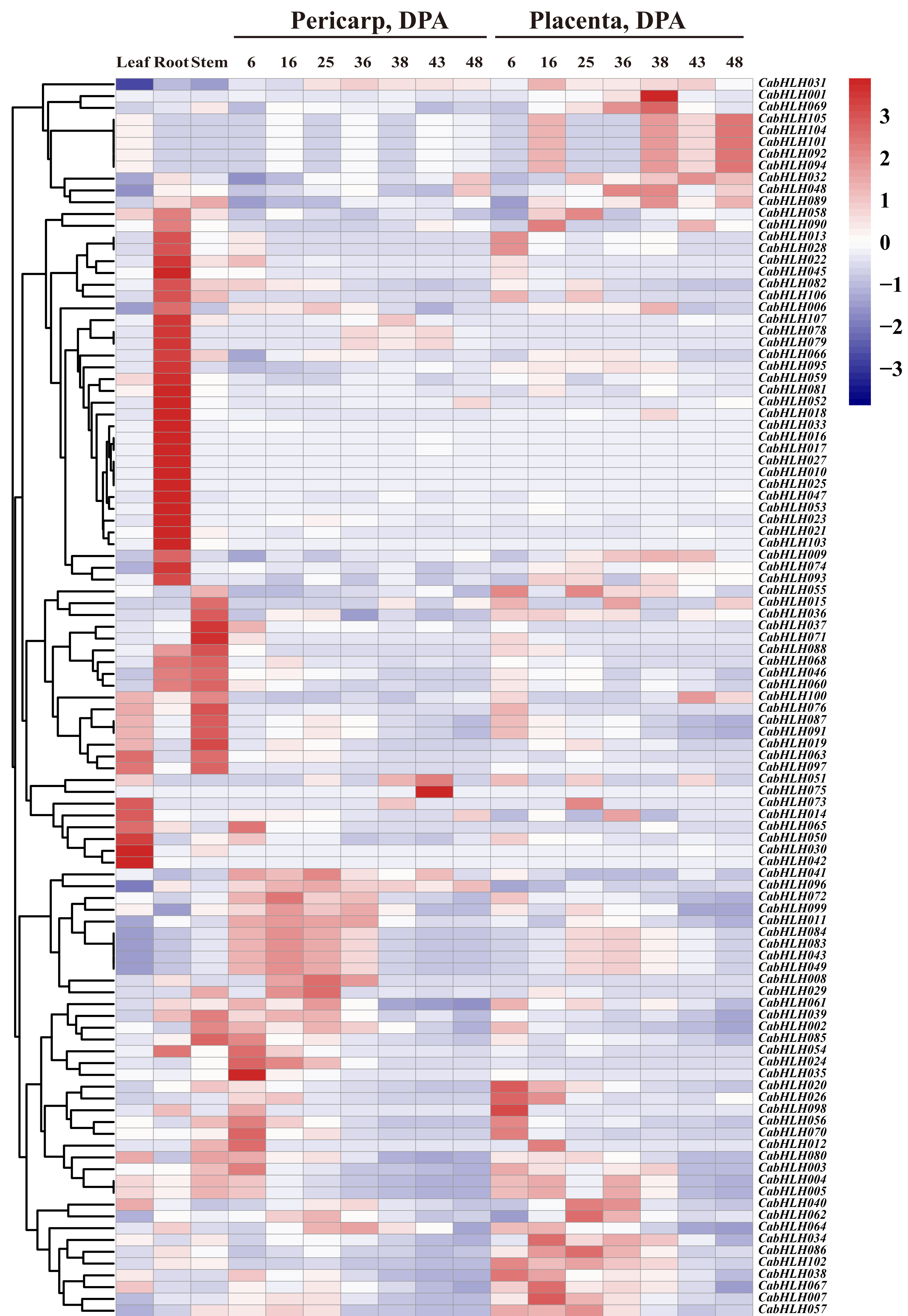


Figure S5. The expression patterns of *CabHLH* genes in different tissues. The RPKM values which was mapped to the *Capsicum annuum* genome of 1.5 version were performed by Kim et al (2014) and used to construct the heat map.

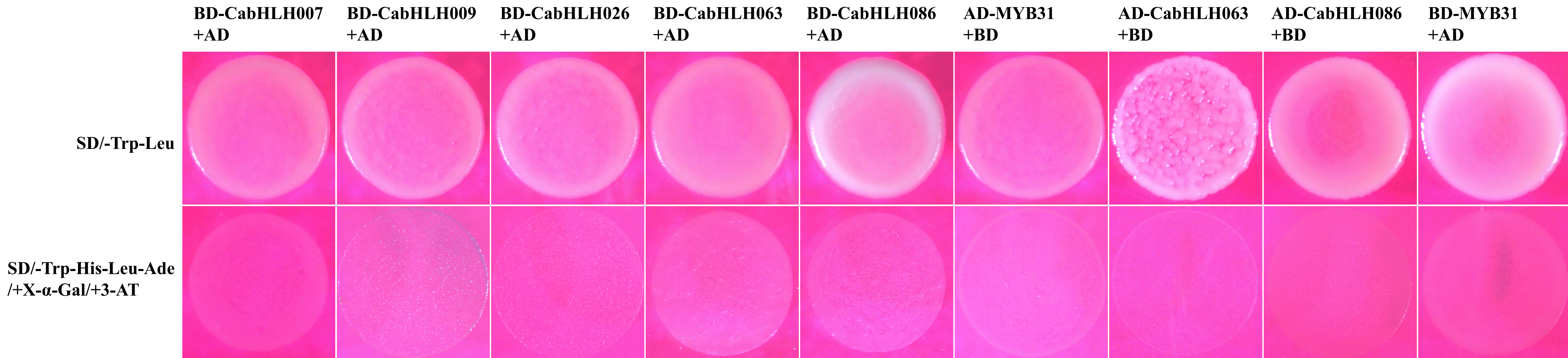


Figure S6. Diagram of yeast two-hybrid assays experiment. AD and BD represent empty pGADT7 and pGBKT7 vectors, separately. Among them, the self-activation function of CabHLH009 and CabHLH026 were inhibited under 15mMol and 30mMol 3-AT, separately. The rest lacked the self-activation function.