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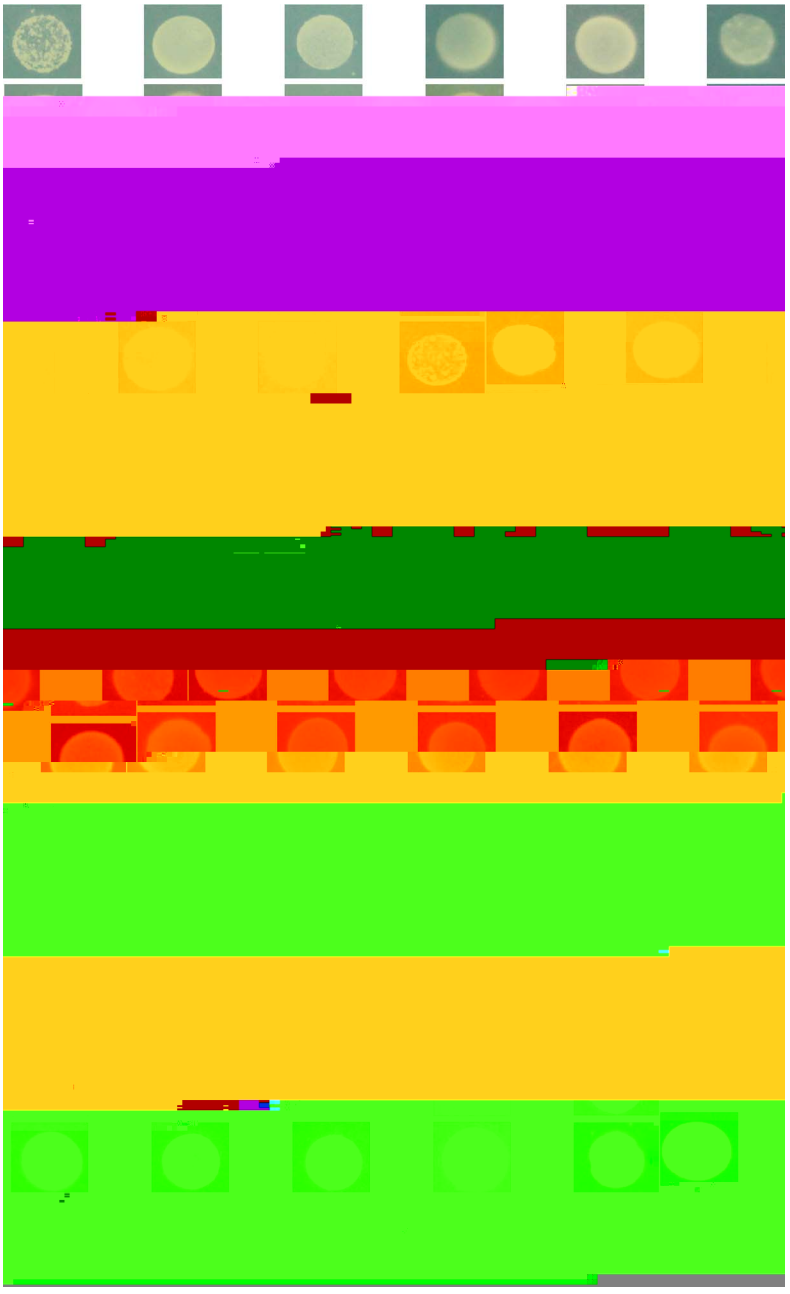

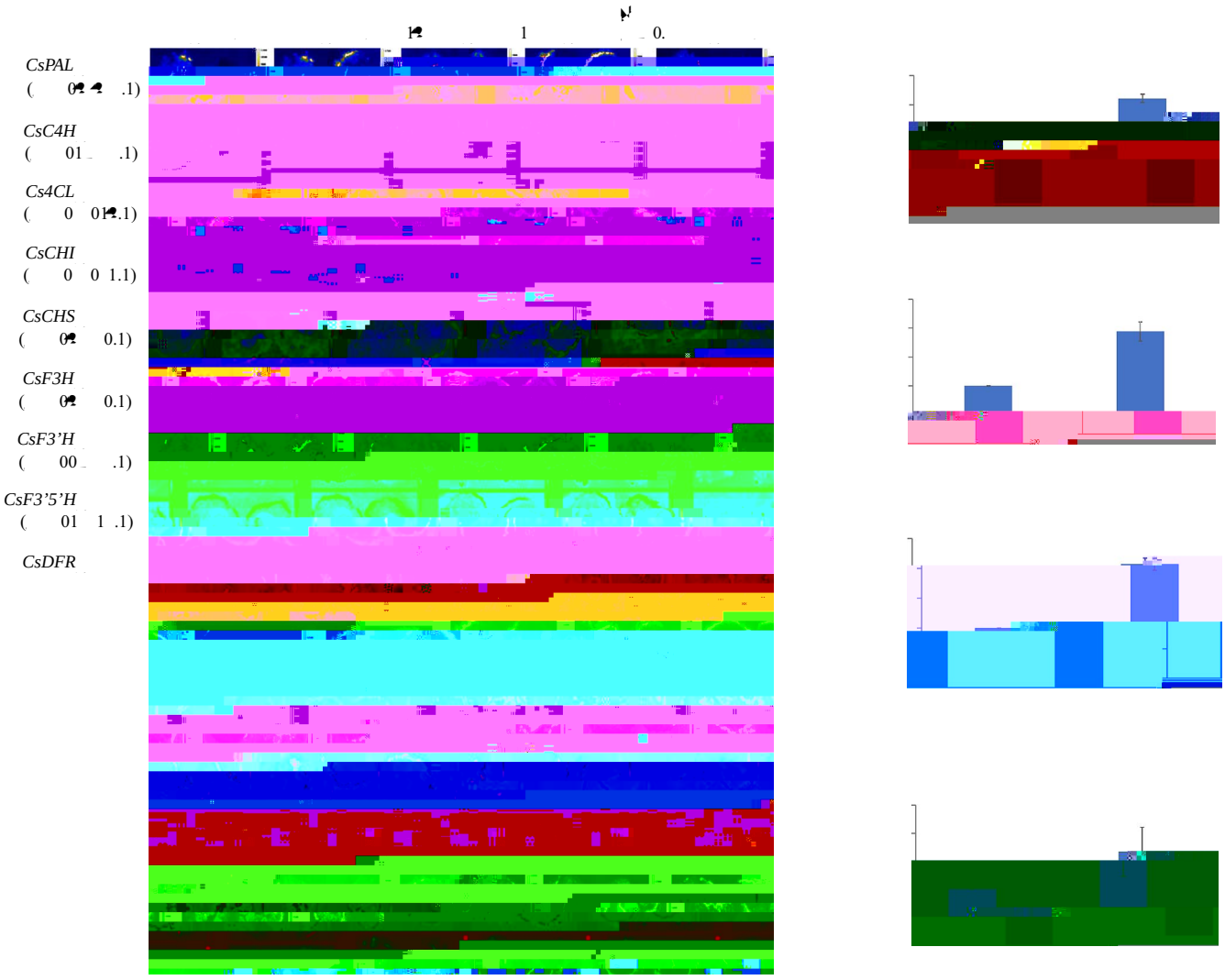
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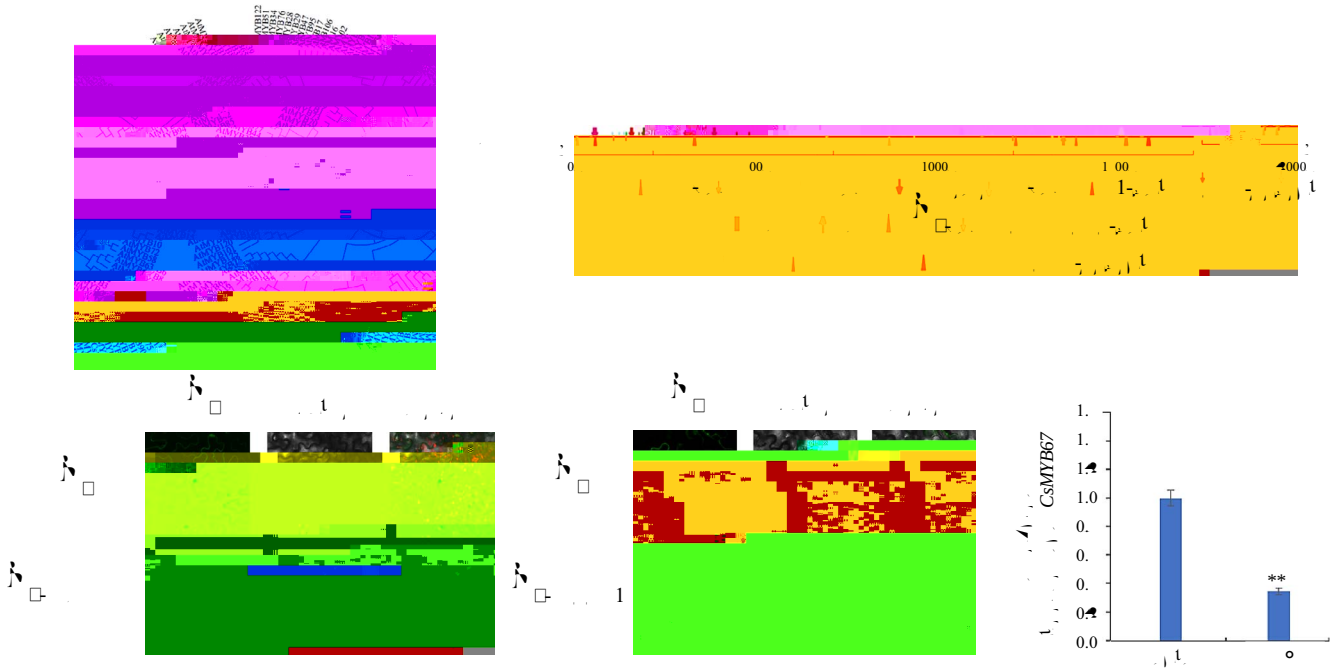
Cs4CL
CsCHI
CsCHS
CsF3H
CsF3'H
CsF3'5'H
CsDFR


0
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1000
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CsMYB67
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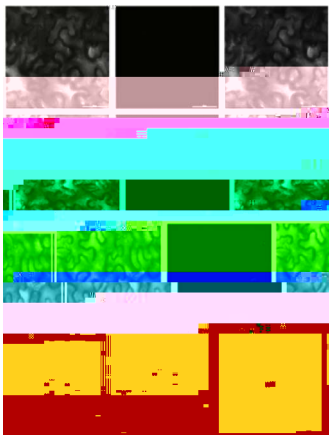

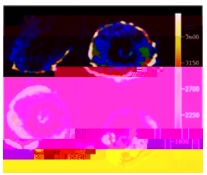

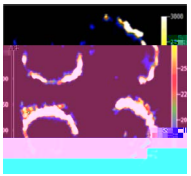

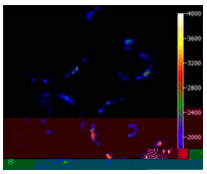

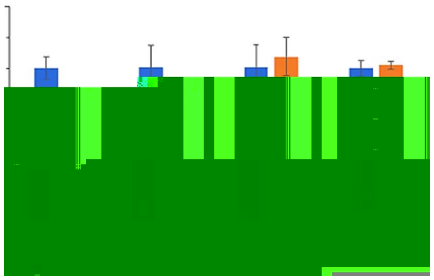

fragments (approximately 2000 bp) of 20 structural genes for flavonoid biosynthesis were cloned. Table S2 (see online supplementary material) lists the primers for cloning. The fragment of the promoter (~2000 bp) was amplified from the cv. '*Fudingdabaicha*' genome DNA by PCR. The PCR program was: 98°C for 2 min, 98°C for 10 s, 60°C for 5 s, 68°C for 20 s, 35 cycles, and 68°C for 2 min. The purified PCR product was ligated into the relevant vector and transformed into *Escherichia coli* DH5α competent cells for sequencing.

Bioinformatic analysis

Cis-acting element prediction

The *cis*-acting elements in the cloned promoters were predicted by the PlantCARE online site at http://bioinformatics.psb.ugent.be/webtools/plantcare/html/.

Prediction of the physicochemical properties of proteins

The ProtParam tool (https://web.expasy.org/protparam/) was employed to predict the physicochemical properties of CsMYB67 and CsTTG1 proteins, including hydrophilicity, molecular mass, and isoelectric point.

Amino acid sequence and phylogenetic analysis methods

Amino acid sequences of MYB transcription factors in *Arabidopsis thaliana* were retrieved from the published database of the *Arabidopsis* Information Resource (TAIR) (https://www.arabidopsis.org/). The construction of phylogenetic tree was performed on the Molecular Evolutionary Genetics Analysis (MEGA) 7.0 software (Mega Limited, Auckland, New Zealand), using the neighbor-joining method (1000 bootstrap replicates).

Subcellular localization

The coding sequences of *CsMYB67* and *CsTTG1* were amplified by PCR and inserted into pCV-GFP-N1 expression vector which contains the green fluorescent protein (GFP) reporter gene. The plasmids were introduced into *Agrobacterium tumefaciens* (GV3101) to select a positive colony for infiltration according to the published method [49]. The construct was transiently expressed in *Nicotiana benthamiana* leaves by the infiltration of *A. tumefaciens* (GV3101). After 44–48 h, the fluorescence signals from tobacco leaf epidermis were examined by a confocal laser scanning electron microscope (Zeiss LSM880).

Effect of temperature on the expression of *CsMYB67*

Because no direct interaction occurred between CsMYB67 and CsHY5, high temperature treatments were employed to investigate the induction of CsMYB67 expression. One-year-old tea seedlings (cv. Fudingdabacha) were placed in the plastic pots and domesticated in the environmental chamber under the following conditions: 25/20°C air temperature (day/night), 600 μmol m^{-2} s^{-1} photosynthetic photo flux density (PPFD), 12 h/12 h photoperiod (day/night) and 80% relative humidity. Then, the tea seedlings were placed at 35°C air temperature in the environmental chamber for 24 h high temperature treatment, and the second leaves were collected, using the second leaves of tea seedlings constantly grown at 25/20°C as control. Five independent biological replicates were collected.

Y1H assay

The coding sequences of *CsMYB4*, *CsMYB12*, *CsMYB67*, *CsC1*, *CsKTN80.4*, and *CsTTG1* were inserted into the pB42AD vectors, respectively, and the promoter fragments of flavonoid biosynthetic genes were constructed into the pLacZi2μ vectors. The Y1H assay was conducted according to the reported method [1]. In brief, the constructed vectors (pLacZi2μ and pB42AD vectors) were co-transformed into the yeast strain EGY48 competent cells, and the empty vectors were co-transformed as the negative control. Then, the yeast cells were cultured on the SD/-Ura/−Trp media (lack of Ura and Trp). After culture at 29°C for 72 hours, the positive colonies were selected and further cultured on the selective SD/Gal/Raf/−Ura/−Trp/+X-gal media plates containing 0.11 M galactose (Gal), 0.02 M raffinose (Raf), 10 × buffered salt (0.26 M $Na_2HPO_4 \bullet 7H_2O$, 0.25 M NaH_2PO_4), 0.1 mM X-gal, and lack of both Ura and Trp.

Transient expression in tobacco system

The coding sequences of *CsHY5*, *CsMYB4*, *CsMYB12*, *CsMYB67*, *CsC1*, *CsKTN80.4*, and *CsTTG1* were inserted into the pGreenII 62-SK vectors as effectors. The promoter fragments of flavonoid biosynthetic genes were constructed into the pGreenII 0800-LUC vectors as reporters. The recombinant pGreenII 62-SK vectors were transformed into GV3101(pSoup-p19) competent cells as effectors, while the empty pGreenII 62-SK vectors were transformed into GV3101 (pSoup-p19) as control. Equal volumes of effectors and reporters were mixed for transient transfection. The control and experimental groups were injected into the same leaf of tobacco (*N. benthamiana*) using 5–6 replicates for each group. After 48 hours (long-day white light illumination), 0.5 mM luciferin (Shanghai Macklin Biochemical Co., Ltd, China) was injected into the infiltrated places and the tobacco leaves were kept in dark for 5 minutes. Afterwards, the fluorescence signals of them were examined by SH-523 Chemiluminescence Imaging System (Shenhua Science Technology Co., Ltd, Hangzhou, China) and the fluorescence intensity was calculated using Image J software (National Institutes of Health, Germany).

Y2H assay

To verify the interaction between CsMYB67 and CsTTG1, the coding sequence of *CsMYB67* was inserted into the pGADT7 vector, and the coding sequence of *CsTTG1* was recombined into the pGBKT7 vector. The constructed vectors (pGADT7-CsMYB67 and pGBKT7-CsTTG1) were co-transformed into the Y2HGold yeast competent cells, and the empty vectors were transformed as negative control. The yeast strains were cultivated on SD/−Leu/−Trp media (lack of Leu and Trp) for 2–3 days, and the positive colonies were selected and adjusted to the OD value of 0.1 for further screening on SD/−Leu/−Trp/-His/+X-α-gal/+AbA [containing 50 ng/mL X-α-gal and 200 ng/mL AbA and lack of Leu, Trp, and Histidine (His)] and SD/−Leu/−Trp/-His/−Ade/+X-α-gal/+AbA [containing 50 ng/mL X-α-gal and 200 ng/mL AbA, and lack of Leu, Trp, His and Adenine (Ade)] selective media plates.

BiFC assay

The recombinant plasmids (CsMYB67-cEYFP and CsTTG1-nEYFP) were constructed by inserting the coding sequence of *CsMYB67* and the coding sequence of *CsTTG1* into the pSAT4A-cEYFP-N1 and pSAT4A-nEYFP-N1 vectors, respectively. The recombinant vectors pSAT4A-cEYFP-N1-CsMYB67 and pSAT4A-nEYFP-N1-CsTTG1 were co-transformed into *A. tumefaciens* GV3101 competent cells, while the empty pSAT4A-cEYFP-N1 vectors and pSAT4A-nEYFP-N1 vectors were co-transformed into GV3101 competent as control. The control and experimental groups were injected into the leaves of tobacco (*N. benthamiana*), using 5–6 replicates for each group. After 48–72 h infiltration, the tobacco

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