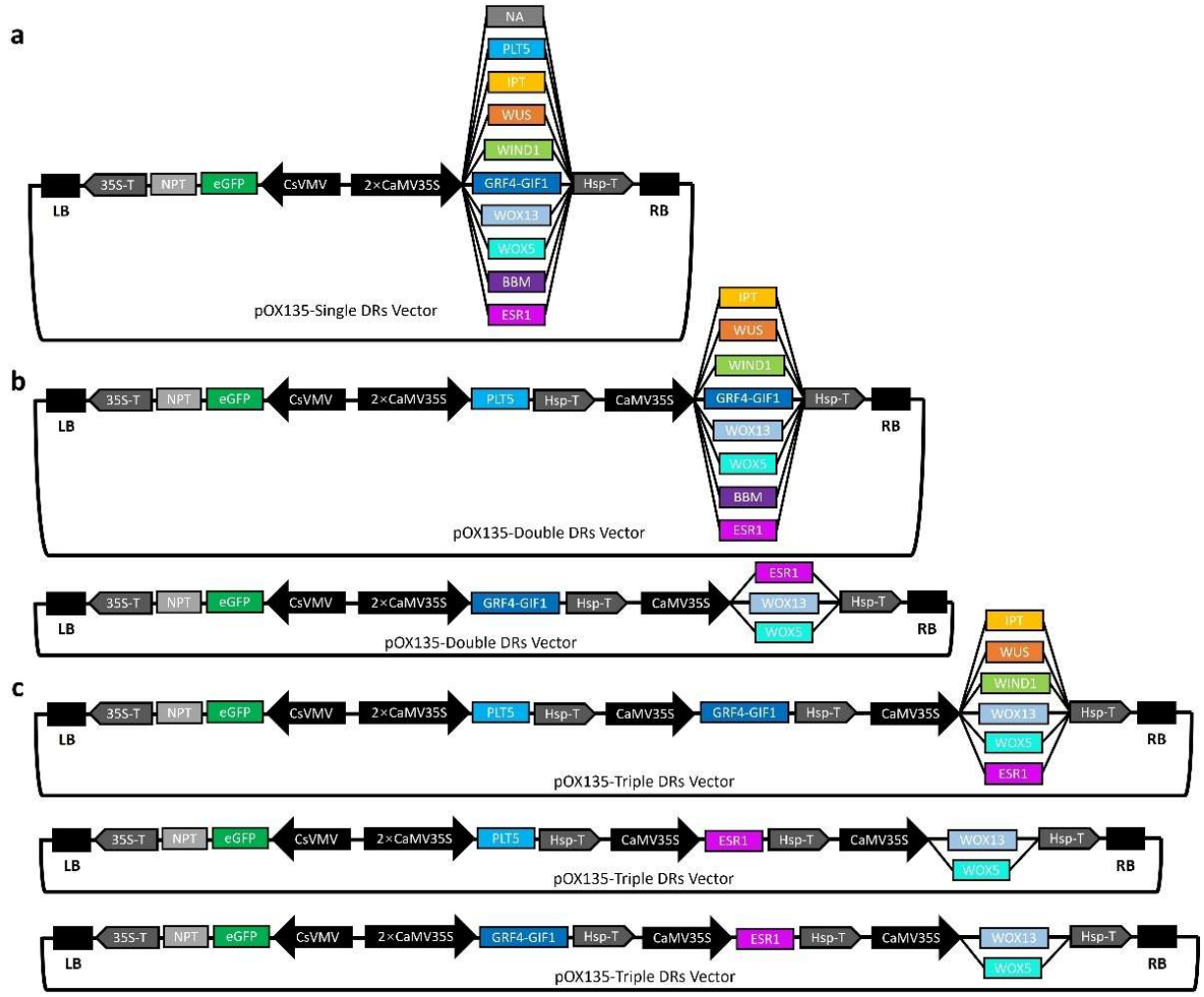
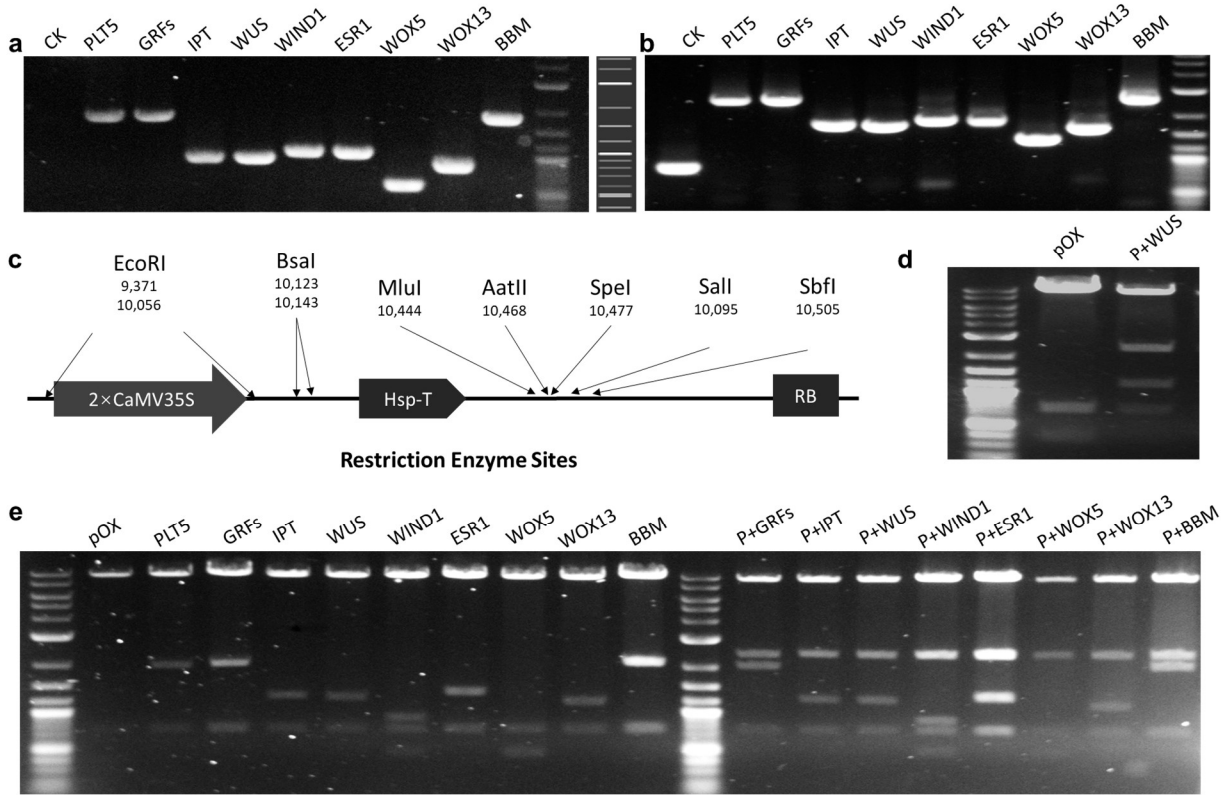
Supplementary Fig.1: Schematic representation of the expression vectors used for evaluating developmental regulators (DRs) in plant transformation.



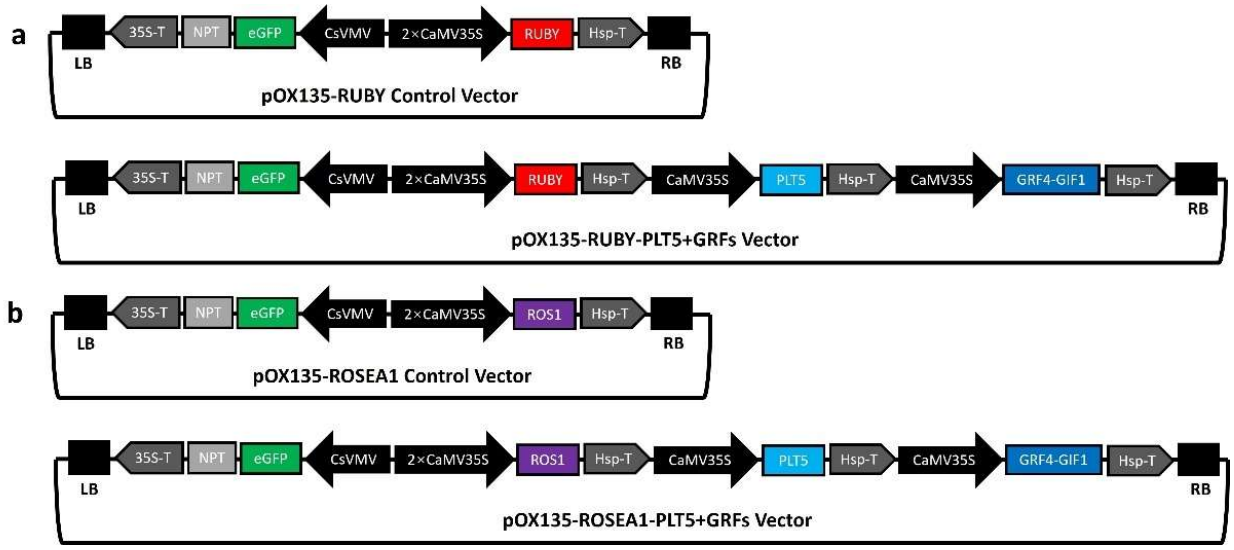
a, Control expression vector pOX135 containing an *eGFP-NPTII* fusion gene driven by the *CsVMV* promoter, without any DRs (NA, labeled as "CK"). Single DR expression vectors are modified pOX135 constructs carrying individual DR genes (*PLT5*, *WIND1*, *IPT*, *WUS*, *GRFs*, *WOX13*, *WOX5*, *BBM*, or *ESR1*), each driven by a double *CaMV35S* promoter (2×*CaMV35S*) (see Supplementary File 2 for promoter sequence information). **b,** Double DR expression vectors are modified pOX135 constructs containing two different DR genes (*PLT5-GRFs*, *PLT5-IPT*, *PLT5-WUS*, *PLT5-WIND1*, *PLT5-ESR1*, *PLT5-WOX5*, *PLT5-WOX13*, *PLT5-BBM*, *GRFs-WOX5*, or *GRFs-WOX13*), each positioned at separate loci and driven by independent 2×*CaMV35S* promoters. **c,** Triple DR expression vectors are modified pOX135 constructs incorporating three distinct DR genes (*PLT5-GRFs-ESR1*, *PLT5-GRFs-WOX5*, *PLT5-GRFs-WOX13*, *PLT5-WUS-WOX5*, *PLT5-ESR1-WOX5*, *PLT5-ESR1-WOX13*, or *GRFs-ESR1-WOX5*), each positioned at separate loci and driven by individual 2×*CaMV35S* promoters. In all constructs, *35S-T* and *Hsp-T* represent terminators. LB and RB indicate the left and right borders of the T-DNA, respectively.

Supplementary Fig. 2: Verification of expression vector construction.



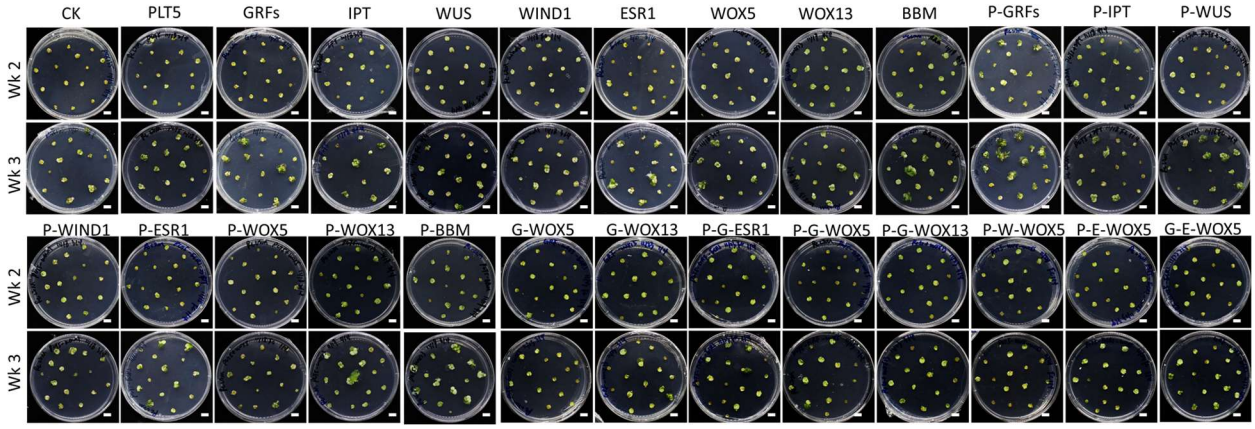
a, PCR amplification of the coding sequences (CDS) of various developmental regulators (DRs) used in this study. Expected amplicon sizes (in bp) are indicated below each lane. **b,** Restriction enzyme digestion of single and double DR expression cassettes to confirm proper vector assembly. Lanes 1-7: single DR cassettes; lanes 8-14: double DR cassettes. Restriction enzymes used were EcoRI, BsaI, MluI, AatII, SpeI, SalI, and SbfI, with their recognition sites shown in the schematic representation in panel c. **c,** Schematic diagram of the restriction enzyme sites used for vector analysis. Hsp-T, heat shock protein terminator; 2×CaMV35S, double Cauliflower Mosaic Virus 35S promoter; RB, right border. **d,** Representative gel image showing the restriction pattern of P-WUS double DR cassette digested with EcoRI, WOX5, and WOX13, confirming the correct ligation of the two DR cassettes. **e,** Restriction enzyme digestion of single and double DR expression vectors using EcoRI, WOX5, and WOX13. Expected band sizes (in bp) are indicated on the left. M, DNA size marker.

Supplementary Fig. 3: Schematic representation of expression vectors containing different visible markers and developmental regulators (DRs).



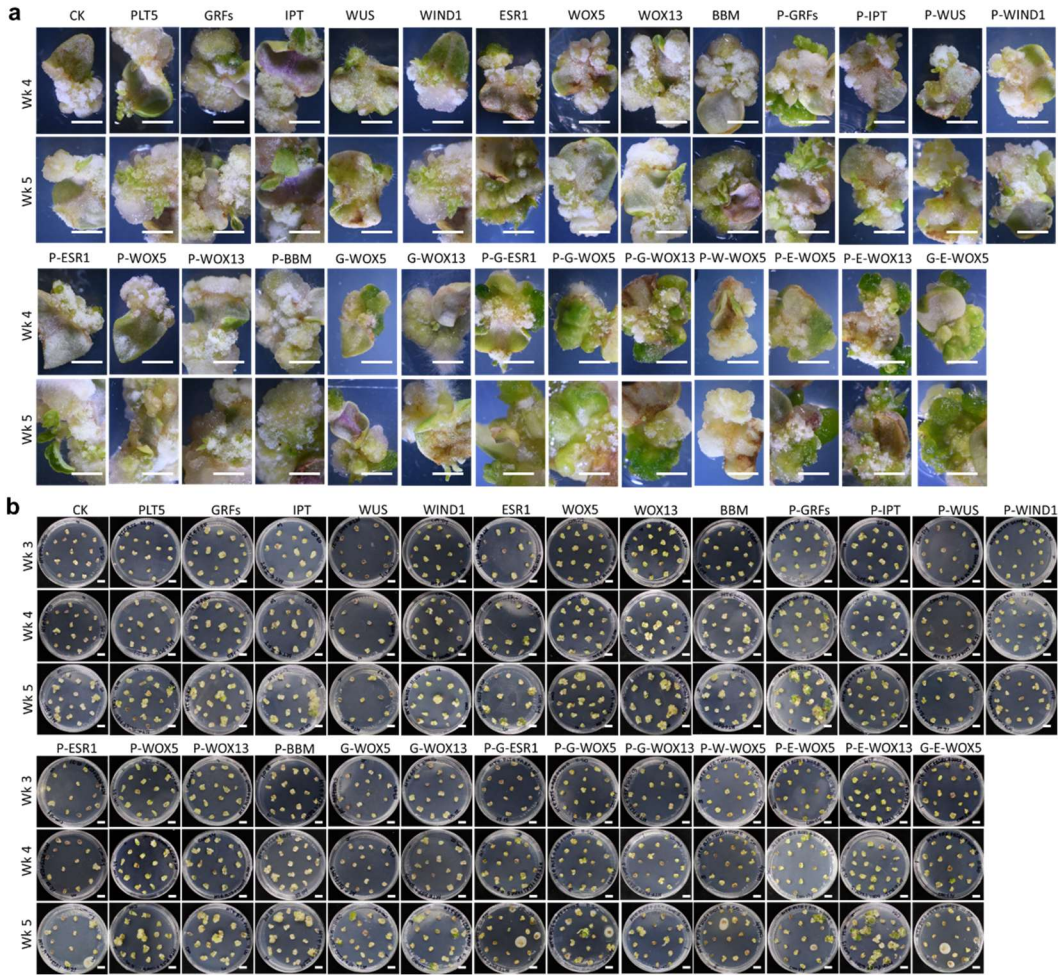
a, pOX135-RUBY control vector harboring an *eGFP-NPTII* fusion gene driven by the *CsVMV* promoter and a synthetic betalain biosynthesis gene (*RUBY*) under the control of a double *CaMV35S* promoter (2×*CaMV35S*). **b,** pOX135-RUBY-PLT5-GRF4/GIF1 (PG+RU) vector, a modified pOX135-RUBY construct incorporating additional cassettes of *PLT5* (from Arabidopsis) and *GRF4*/*GIF1* (from grape), both driven by 2×*CaMV35S* promoters. **c,** pOX135-ROSEA1 control vector containing an *eGFP-NPTII* fusion gene driven by the *CsVMV* promoter and an anthocyanin regulatory gene (*ROSEA1*) under the control of 2×*CaMV35S*. **d,** pOX135-ROSEA1-PLT5-GRF4/GIF1 (PG+RO) vector, a modified pOX135-ROSEA1 construct incorporating additional cassettes of *PLT5* (from Arabidopsis) and *GRF4*/*GIF1* (from grape), both driven by 2×*CaMV35S* promoters. In all constructs, *35S-T* and *Hsp-T* represent terminators, while LB and RB indicate the left and right borders of the T-DNA, respectively.

Supplementary Fig. 4: Comprehensive screening of DRs effects on callus induction, shoot regeneration, and transformation efficiency in *Petunia hybrida*.



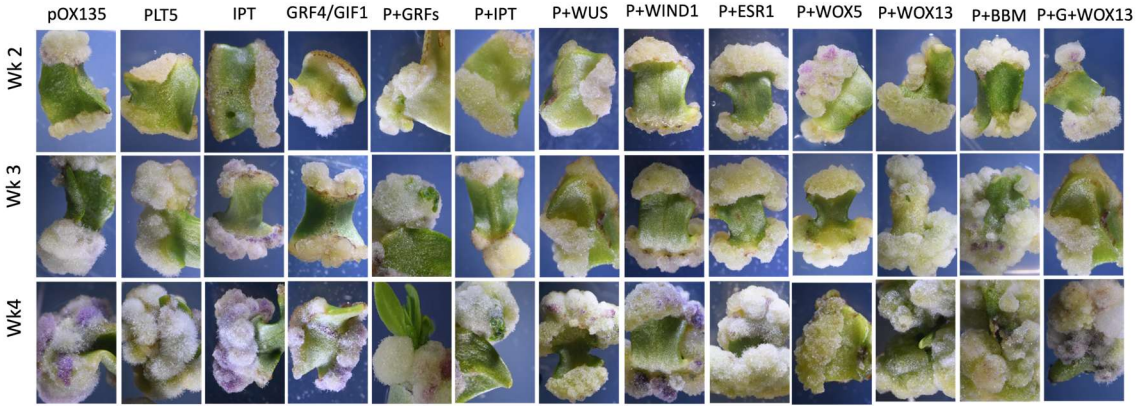
Representative images of Petunia culture plates showing leaf explant responses to various DRs on callus-induction MS medium at 2- and 3- wpi. Upper panels show individual DRs (pOX135 control, GRFs, IPT, WUS, WIND1, ESR1, WOX5, WOX13, BBM); middle panels display PLT5-based combinations (PLT5, P-GRFs, P-IPT, P-WUS, P-WIND1, P-ESR1, P-WOX5, P-WOX13, P-BBM); and lower panels present more complex combinatorial treatments (G-WOX5, G-WOX13, P-G-ESR1, P-G-WOX5, P-G-WOX13, P-W-WOX5, P-E-WOX5, P-E-WOX13, G-E-WOX5) (where P = PLT5, G = GRF4/GIF1, E = ESR1, W = WUS). Images were selected to represent the average response across three biological replicates, with each replicate consisting of 20 leaf explants cultured under identical conditions.

Supplementary Fig.5: Comprehensive screening of DRs for regeneration in tomato of DR effects on callus induction, shoot regeneration, and transformation efficiency in tomato (*Solanum lycopersicum* cv. Micro-Tom).



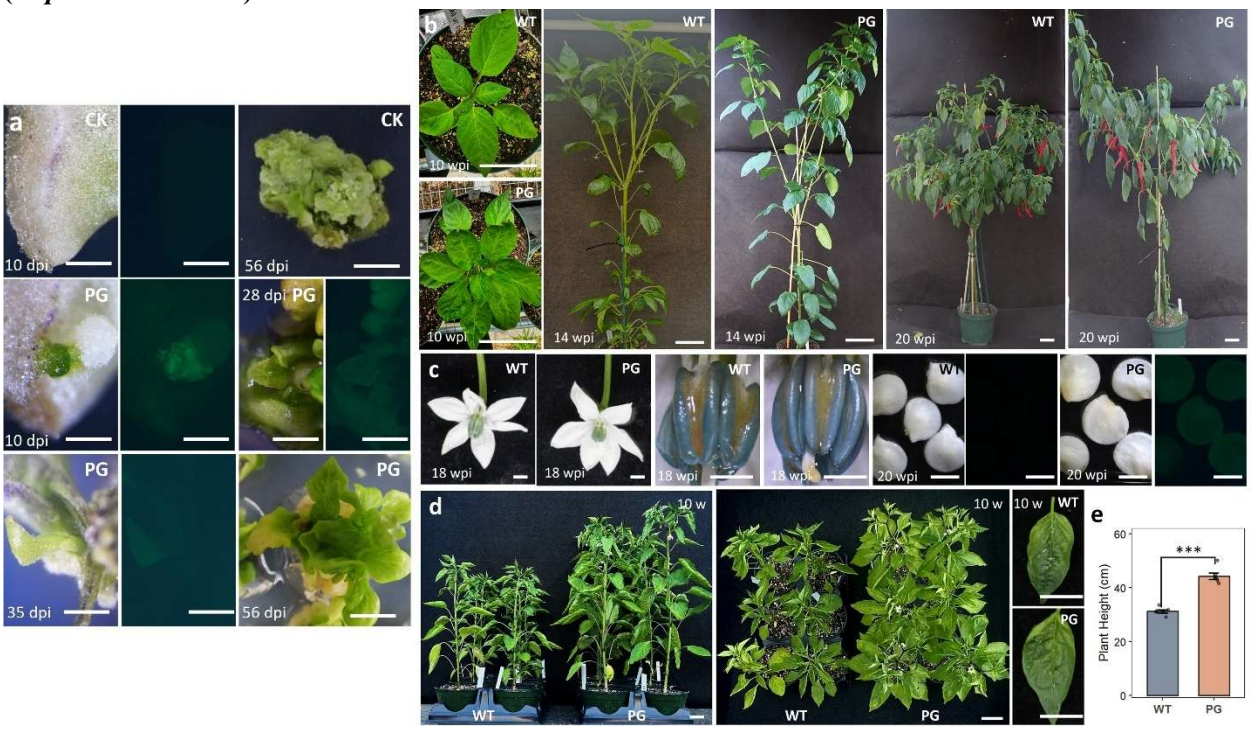
a, Representative images of individual tomato explants illustrating regeneration responses to various DR combinations at 3 (See Fig.1a), 4, and 5 wpi. Upper panels show individual DRs (pOX135 control, GRFs, IPT, WUS, WIND1, ESR1, WOX5, WOX13, BBM); middle panels display PLT5-based combinations (PLT5, P-GRFs, P-IPT, P-WUS, P-WIND1, P-ESR1, P-WOX5, P-WOX13, P-BBM); and lower panels present more complex combinatorial treatments (G-WOX5, G-WOX13, P-G-ESR1, P-G-WOX5, P-G-WOX13, P-W-WOX5, P-E-WOX5, P-E-WOX13, G-E-WOX5) (where P = PLT5, G = GRF4/GIF1, E = ESR1, W = WUS). **b,** Representative images of tomato culture plates showing cotyledon explant responses to various DR combinations on MS medium at 3-, 4-, and 5- wpi. Images were selected to represent the average response across three biological replicates, with each replicate consisting of 20 cotyledon explants cultured under identical conditions. Statistical analysis of the DR evaluation on tomatoes is provided in Supplementary Table 1.

Supplementary Fig.6: Comparative analysis of developmental regulator (DR) effects on regeneration in chili pepper (*Capsicum annuum*).



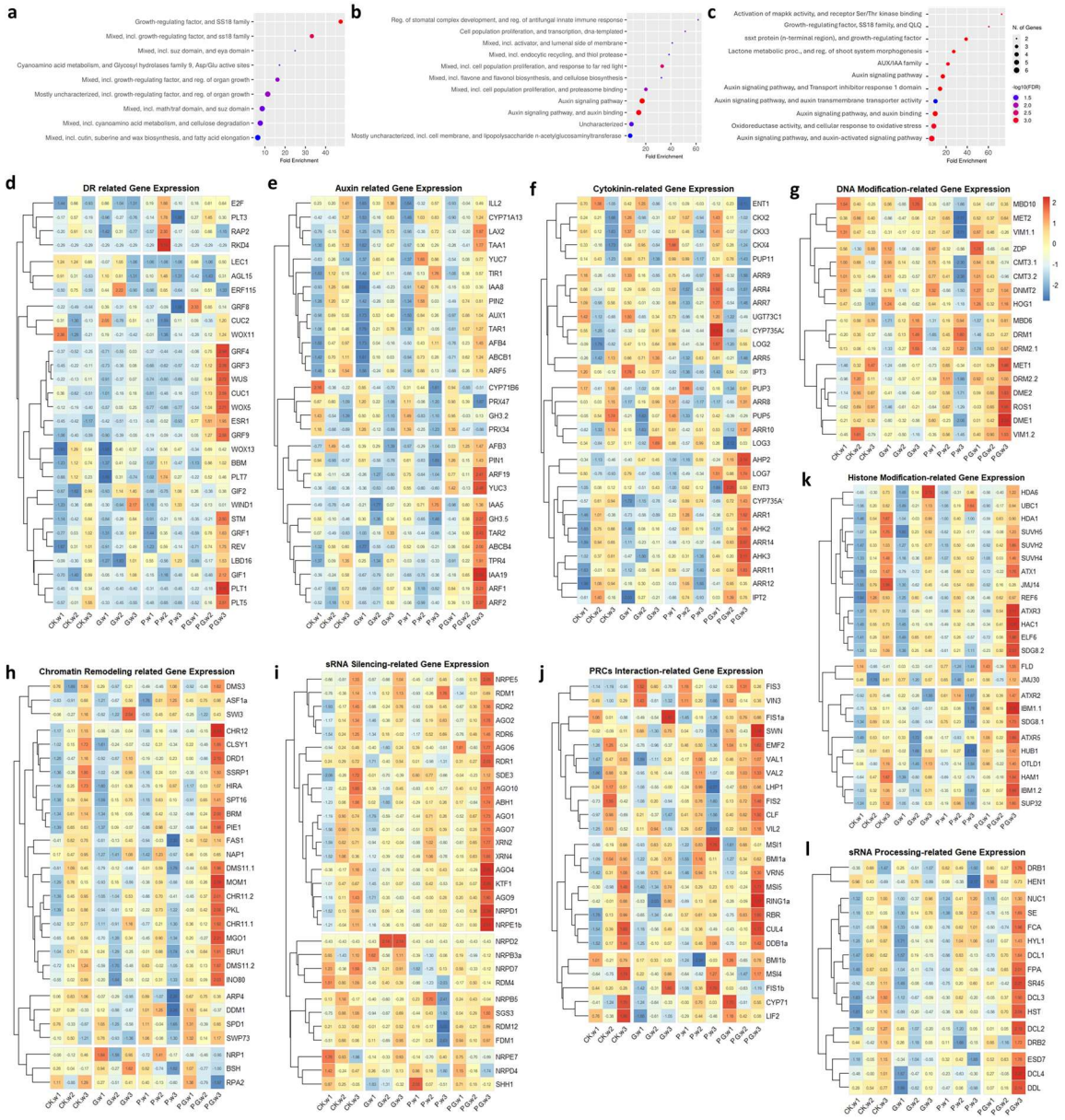
Representative images of individual chili pepper cotyledon explants showing regeneration responses following Agrobacterium-mediated transformation with various DR constructs at 2-, 3-, and 4 wpi. The tested constructs include pOX135 (control), PLT5, IPT, GRF4/GIF1, P-GRFs, P-IPT, P-WUS, P-WIND1, P-ESR1, P-WOX5, P-WOX13, P-BBM, and P-G-WOX13 (where P = PLT5, G = GRF4/GIF1). Images were selected from full culture plates to represent the average response across three biological replicates, with each replicate consisting of 20 cotyledon explants cultured under identical conditions. Statistical analysis of the DR evaluation on chili is provided in Supplementary Table 3.

Supplementary Fig.7: Phenotypic characterization of *PLT5-GRFs* transgenic chili pepper (*Capsicum annuum*).



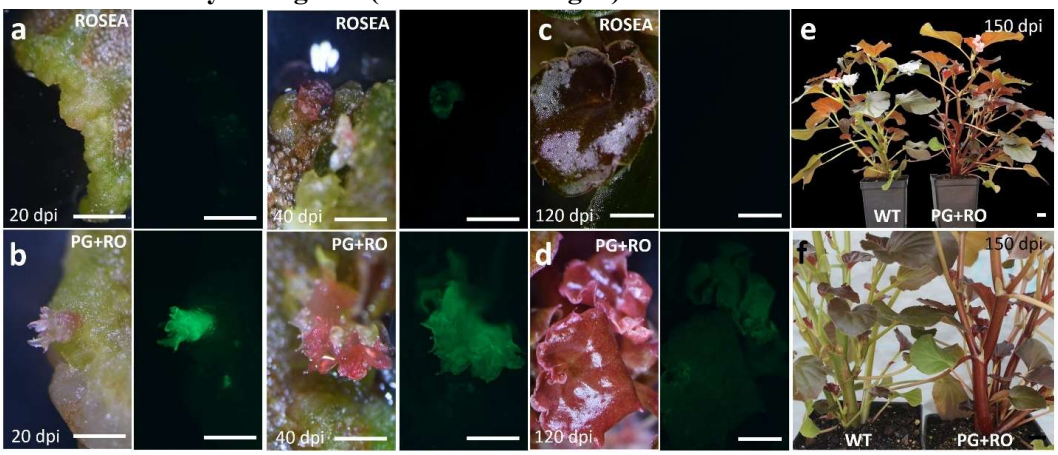
a, Fluorescence microscopy of cotyledon explants and calli at 10, 28, 35, and 56 dpi with control (CK) or *PLT5-GRF4* (PG) constructs. Insets show GFP expression in transgenic PG shoots. Scale bars, 2 mm. **b,c,** Developmental comparison of T0 transgenic PG and wild-type (WT) chili pepper plants at various growth stages: seedlings at 10 weeks post-inoculation (wpi) (b, left), plants at 14 and 20 wpi (b, middle and right), flowers at 18 wpi (c, left), and seeds at 20 wpi (c, middle). GFP fluorescence in WT and PG seeds is shown in c, right. Scale bars, 1 cm (b), 5 mm (c, left and middle), and 2 mm (c, right). **d,** Side view (top) and top view (bottom) of T1 transgenic PG and WT chili pepper plants at 10 weeks after seed germination, showing differences in plant architecture and leaf morphology. Scale bars, 5 cm. **e,** Quantification of plant height in WT and PG plants at 10 weeks after seed germination. Data are presented as mean ± s.d. (n = 6 plants per genotype, 3 biological replicates). Asterisks indicate statistically significant differences based on a two-tailed Student's t-test (***P < 0.001).

Supplementary Fig.8: Transcriptional profiling reveals *PLT5* and *GRF*s-mediated gene expression changes associated with enhanced transformation efficiency in *Capsicum annuum*.



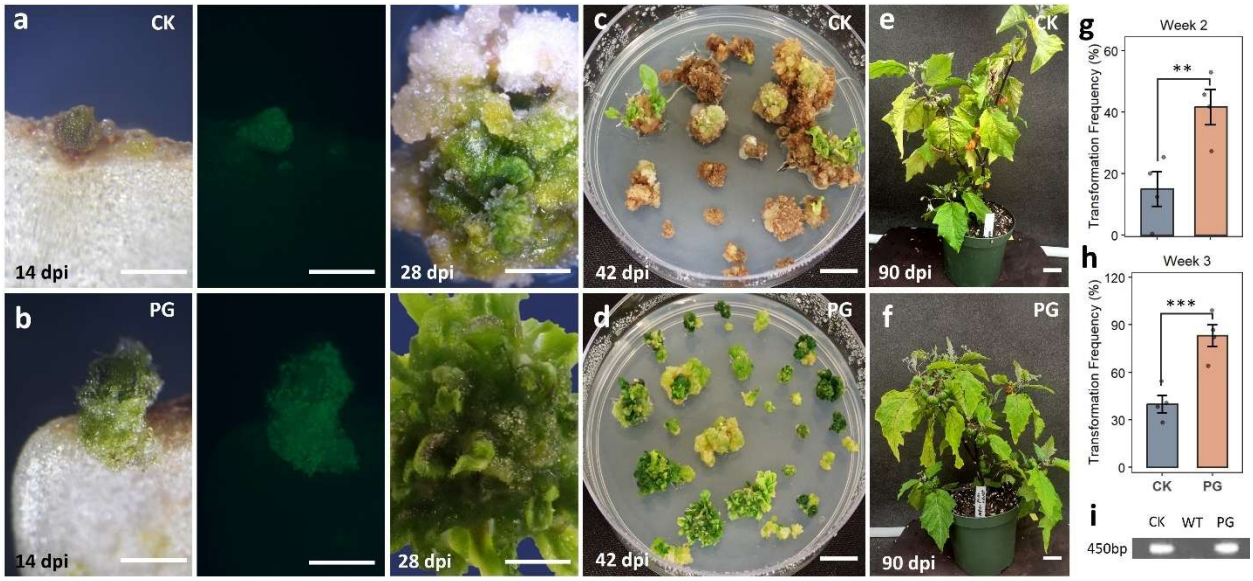
a-c, KEGG pathway enrichment analysis of differentially expressed genes (DEGs) in *C. annuum* at 3 wpi with *PLT5-GRFs* (PG) compared to the control (CK). The analysis was performed using homologs of *C. annuum* DEGs identified in *Arabidopsis thaliana* (a), *Nicotiana tabacum* (b), and *Solanum lycopersicum* (c). Dot size represents the number of DEGs in each pathway, and color indicates the statistical significance (−log10(false discovery rate)). **d–l,** Hierarchical clustering and heatmap visualization of transcriptional profiles for genes involved in key regulatory pathways: developmental regulation (d), auxin signaling (e), cytokinin signaling (f), DNA modification (g), histone modification (k), chromatin remodeling (h), small RNA silencing (i), Polycomb Repressive Complex (PRC) interactions (j), and small RNA processing (l). Expression levels are presented as log2(transcripts per million + 1) values across three biological replicates for each treatment: CK, PLT5 alone (P), GRF4 alone (G), and PG at 1-, 2-, and 3- wpi. Color scale represents normalized expression levels from low (blue) to high (red).

Supplementary Fig. 9: Co-expression of *PLT5* and *GRF*s enhances anthocyanin accumulation and transformation efficiency in Begonia (continued in Fig. 5).



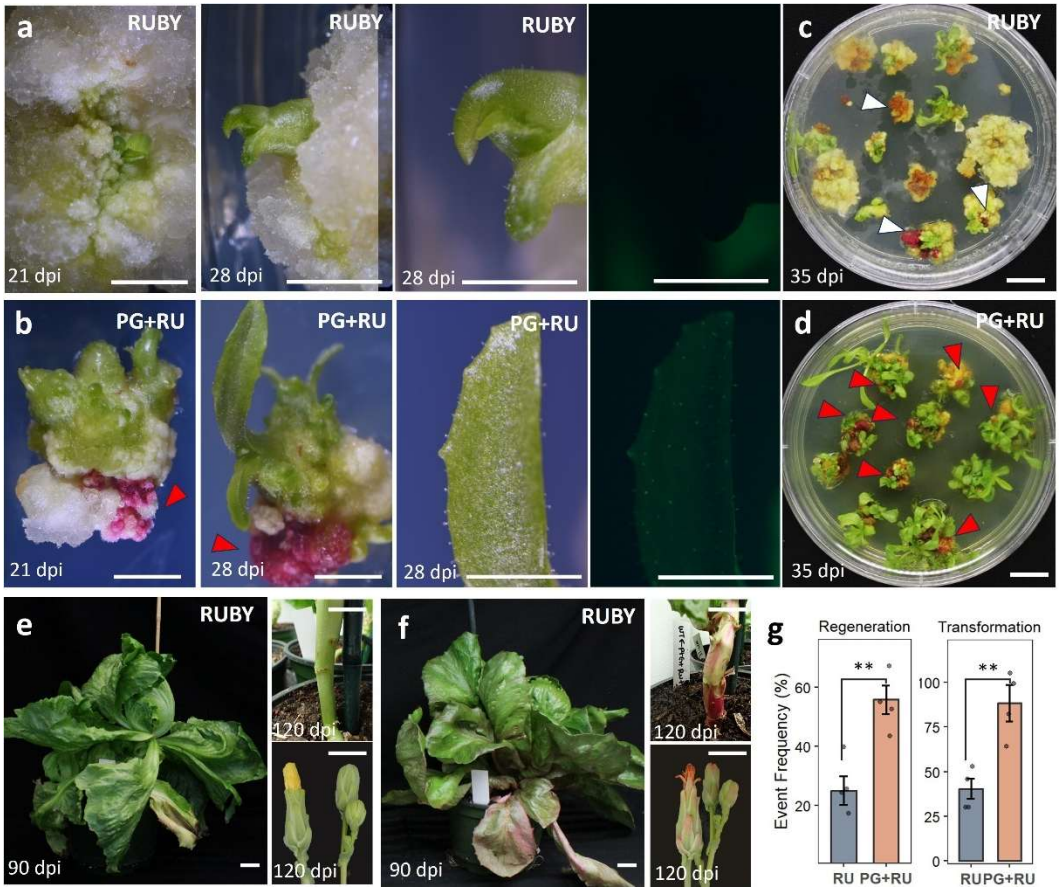
a,b, Leaf explants transformed with the *ROSEA1* control plasmid (a) or the *PLT5-GRFs+ROSEA1* (PG+RO) plasmid (b) showing callus development, GFP fluorescence, and red pigmentation at 20 and 40 dpi. PG+RO explants exhibit enhanced GFP fluorescence and intense red pigmentation compared to the control. **c,d,** Phenotypic comparison of wild-type (WT) (c) and transgenic PG+RO (d) plants at 120 dpi, showing detailed views of leaves under bright field (left) and UV illumination (right). Scale bars, 1 cm (a-d). **e,f,** Phenotypic comparison of WT (e) and PG+RO (f) plants at 150 dpi, displaying whole plant morphology (e) and anthocyanin accumulation in leaves and stems (f). PG+RO plants exhibit enhanced anthocyanin production compared to WT. Scale bars, 2 cm (e, f).

Supplementary Fig.10: Co-expression of *PLT5* and *GRFs* enhances shoot regeneration and transformation efficiency in *Solanum aethiopicum*.



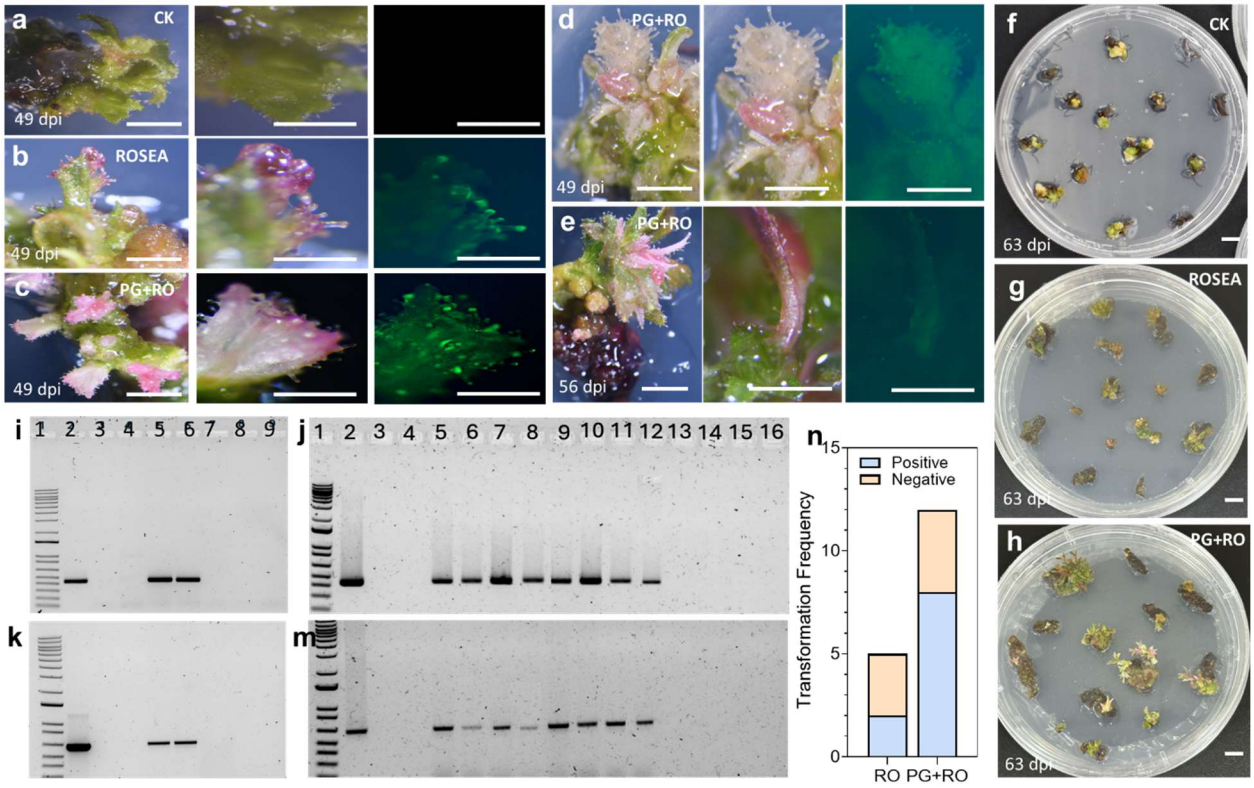
a, b, Fluorescence microscopy of calli at 14- and 28- dpi with control (CK) or *PLT5-GRF4* (PG) constructs. **c, d,** Regenerated shoots at 42- dpi from CK (c) and PG (d) treatments. **e, f,** Transgenic plantlets at 90 dpi from CK (e) and PG (f) treatments. Scale bars, 5 mm. **g, h,** Transformation frequency (percentage of explants producing transgenic shoots) in CK and PG treatments at 2 weeks (g) and 3 weeks (h) post-transformation. Data are presented as mean ± s.d. (n = 4 biological replicates, each consisting of 15 explants). Asterisks indicate statistically significant differences based on a two-tailed t-test (*P < 0.05; **P < 0.01; ***P < 0.001). **i,** PCR detection of the *GFP* transgene (450 bp) in T0 transgenic seedlings from CK and PG treatments. Wild-type (WT) plants served as a negative control.

Supplementary Fig.11: PLT5 and GRF co-expression enhances regeneration and transformation efficiency in lettuce (*Lactuca sativa*).



a,c,e, Developmental progression of cotyledon explants transformed with the control vector pOX135-RUBY (RU) from 21 to 120 days post-inoculation (dpi). RU explants developed calli with weak GFP fluorescence and red pigmentation. Representative shoots at 28 dpi (c, white arrowhead) show limited GFP fluorescence. Shoot at 35 dpi (e) and fully regenerated RU plants at 90 and 120 dpi exhibited normal green phenotypes without noticeable detectable pigment accumulation in vegetative or reproductive tissues. The representative culture plate at 35 dpi (c, top right) demonstrates the limited shoot regeneration and transformation frequency of the RU construct. **b,d,f,** Developmental progression of cotyledon explants transformed with the *PLT5-GRFs+RUBY* (PG+RU) vector from 21 to 120 dpi. PG+RU explants showed strong GFP fluorescence and red pigmentation. Representative shoots at 28 dpi (d, red arrowhead) and displays strong GFP fluorescence. Fully regenerated PG+RU plants at 90 and 120 dpi exhibited strong red pigmentation in leaves, stems, and flowers. The representative culture plate at 35 dpi (d, bottom right) demonstrates the enhanced shoot regeneration and transformation frequency of the PG+RU construct. Red arrowheads denote the regenerated shoots from PG+RU, while white arrowheads indicate those from RU. Scale bars, 2 mm (a,b), 1 mm (c,d), 1 cm (e,f). **g,** Regeneration frequency (percentage of explants producing shoots) and transformation frequency (percentage of explants producing transgenic shoots) in RU and PG+RU treatments at 4 wpi. Data are presented as mean ± s.d. (n = 3 biological replicates, each consisting of 15 explants). Asterisks indicate statistically significant differences based on a two-tailed Student's t-test (**P < 0.01).

Supplementary Fig. 12: Co-expression of PLT5 and GRFs enhances genetic transformation efficiency in blueberry using the *ROSEA* visible marker.



a–**c** Representative shoot-regenerating explants of *Vaccinium corymbosum* 'Albus' transformed with pOX135 empty vector (CK) (**a**), pOX135-*ROSEA* (ROSEA) (**b**) and pOX135-PG+RO (PLT5+GRFs+*ROSEA1*) (**c**) at 49 days post inoculation (dpi), shown under bright field (left) and fluorescence (right). **d**, **e** Additional pOX135-PG+RO explants at later time points (49 and 56 dpi, as indicated), highlighting pink anthocyanin pigmentation and GFP fluorescence in emerging shoots. **f**–**h** Whole regeneration plates at 63 dpi for CK (**f**), ROSEA (**g**) and PG+RO (**h**). **i**, **k** PCR detection of the *AmROSEA1* transgene (450 bp) in independent transformants. **j**, **m** PCR detection of the *eGFP* transgene (630 bp) in the same samples. For each gel, lane 1, 1 kb Plus DNA ladder; lane 2, plasmid positive control (pOX135-*ROSEA1* in **i**–**j**; pOX135-PG+RO in **k**–**m**); lane 3, water negative control; lane 4, wild-type 'Albus'; lanes 5–9 (**i**–**j**) or 5–11 (**k**–**m**), independent putative transformants. **n** Transformation frequency of each construct calculated as the percentage of PCR-positive shoots among total regenerated shoots. Scale bars, as indicated.

Supplementary Table 1: Effects of developmental regulators (DRs) on plant regeneration and transformation in *Petunia hybrida*.

Developmental Regulator (DRs)	2 wpi			3 wpi		
	Callus induction frequency (%)	Shoot induction frequency (%)	Transformation frequency (%)	Callus induction frequency (%)	Shoot induction frequency (%)	Transformation frequency (%)
CK	56.03±8.86	4.6±3.99	4.6±3.99	69.84±7.65	16.35±4.42	23.71±5.25
PLT5	69.58±3.41	10.83±3.63	13.06±6.68	78.19±4.26	32.64±6.77*	42.64±0.07*
GRFs	54.6±20.12	2.38±4.12	2.38±4.12	74.13±15.26	20.79±6.21	30.00±9.37
IPT	68.19±5.44	6.39±0.24	6.39±0.24	70.56±18.73	19.31±7.1	25.83±12.27
WUS	44.86±11.9	8.47±3.49	8.47±3.49	59.58±3.15	19.03±5.84	25.28±12.09
WIND1	68.19±5.44	15±4.33*	19.31±7.1*	78.89±6.74	29.72±6.79*	40.42±9.38*
ESR1	58.18±3.15	5.25±4.71	8.28±9.2	68.89±7.7	31.11±10.18	35.56±10.18
WOX5	61.25±15.52	13.19±6.88	15.42±7.94	72.08±13.71	26.25±7.3	32.92±13.96
WOX13	61.11±11.71	6.53±6.67	6.53±6.67	71.25±12.37	24.03±8.08	32.78±7.52
BBM	60.97±10.8	10.83±3.63	15.14±7.36	62.22±19.25	26.67±6.67	37.78±7.70*
PLT5-GRFs	72.7±1.1*	25.08±4.5**	41.11±8.39**	84.44±3.85*	53.33±6.67**	80.44±5.85**
PLT5-IPT	68.75±22.53	12.5±6.25	16.67±9.55	83.33±9.55	33.33±3.61**	50.00±6.25**
PLT5-WUS	61.27±10.62	20.48±6.72*	29.52±3.43**	72.86±5.97	45.56±5.09**	61.43±7.11**
PLT5-Wind1	53.13±4.42	3.13±4.42	3.13±4.42	56.25±0	25±6.25	35.42±7.22
PLT5-ESR1	41.39±10.55	2.08±3.61	2.08±3.61	53.33±9.43	23.33±4.71	33.33±9.43
PLT5-WOX5	68.75±17.68	3.13±4.42	3.13±4.42	75±8.84	21.88±4.42	34.38±4.42*
PLT5-WOX13	61.11±11.71	4.31±3.73	6.39±6.25	76.39±12.48	25.97±5.66	36.94±3.37*
PLT5-BBM	53.33±6.67	13.33±0*	17.78±3.85*	54.58±11.51	34.86±4.57**	48.06±9.14*
GRFs-WOX5	48.31±8.69	7.64±8.42	7.64±8.42	57.24±1.05	26.59±6.11	28.97±4.18
GRFs-WOX13	60.02±5.77	9.33±2.81	9.33±2.81	58.12±12.49	24.52±4.31	31.21±4.52
P-GRFs-ESR1	35.81±6.19	8.91±3.86	10.99±3.36	39.98±5.77	19.76±5.36	32.84±11.17
P-GRFs-WOX5	18.25±4.32	6.83±6.67	6.83±6.67	29.52±3.43	11.27±3.57	17.94±9.92
P-GRFs-WOX13	44.27±3.71	11.79±4.56	16.58±5.63	40±13.33	15.56±3.85	22.22±3.85
P-WUX-WOX5	26.67±6.67	4.44±3.85	4.44±3.85	26.67±6.67	8.89±3.85	13.33±6.67
P-ESR1-WOX5	15.56±3.85	4.44±3.85	4.44±3.85	20.48±6.72	6.83±0.27	9.05±3.72*
G-WOX5-ESR1	43.47±16.67	8.61±3.37	10.69±6.98	47.92±17.05	19.72±7.09	26.25±11.92

Note: Cotyledon explants (n = 20) were cultured on callus-induction MS medium supplemented with 1 mg/L 6-benzylaminopurine (BAP), 0.1 mg/L 1-naphthaleneacetic acid (NAA), 100 mg/L kanamycin, and 100 mg/L Timentin for 2 and 3 weeks to evaluate the effects of various DRs on plant regeneration and transformation. Callus induction frequency (%), shoot induction frequency (%), and transformation frequency (%) were calculated as follows: callus induction frequency (%) = (number of explants with calli / total number of explants) × 100; shoot induction frequency (%) = (number of explants with shoots / total number of explants) × 100; transformation frequency (%) = (total number of shoots expressing GFP / total number of explants) × 100. Data are presented as mean ± s.d. from three independent experiments. Asterisks indicate statistically significant differences compared to the control (CK) based on a two-tailed Student's t-test (*P < 0.05; **P < 0.01).

Supplementary Table 2: Effects of DRs on plant regeneration and transformation in tomato (*Solanum lycopersicum* cv. Micro-Tom).

Developmental Regulator (DRs)	3 wpi			4 wpi			5 wpi		
	Callus induction frequency (%)	Shoot induction frequency (%)	Transformation frequency (%)	Callus induction frequency (%)	Shoot induction frequency (%)	Transformation frequency (%)	Callus induction frequency (%)	Shoot induction frequency (%)	Transformation frequency (%)
CK	26.56±9.38	0	0	37.5±8.84	4.69±3.13	4.71±3.13	45.31±9.38	7.81±3.13	10.94±5.98
PLT5	33.33±12.17	3.33±3.85	3.33±3.85	48.54±17.16	9.69±3.75	12.92±3.93*	54.69±16.08	12.92±5.46	27.81±6.52*
GRFs	30±8.61	0	0	38.46±11.61	3.59±4.17	5.23±4.10	48.72±14.19	10.26±3.58	13.59±9.2
IPT	47.39±9.89*	3.57±7.14	3.57±7.14	58.24±6.23**	8.57±6.64	8.06±6.01	57.13±11.64	6.79±5.45	8.45±8.36
WUS	41.79±8.63	0	0	44.32±5.72	6.14±0.59	6.14±0.59	57.95±6.82	15.72±8.91	17.99±13.04
WIND1	51.47±2.94**	5.88±4.8*	8.82±7.59	63.24±7.4**	8.82±3.4	9.07±3.12	69.12±7.4**	19.12±5.63*	27.94±10.05*
ESR1	45.31±13.86	3.13±3.61	4.69±5.98	56.25±15.77	14.58±4.17**	20.83±14.43*	70.83±10.76*	33.33±6.8**	40.25±10.49**
WOX5	39.71±7.4	1.47±2.94	1.47±2.94	61.76±7.59**	5.88±4.8	4.94±5.77	77.94±8.82**	20.59±5.88**	29.41±11.76*
WOX13	50±7.59**	0	0	55.88±7.59*	7.42±1.84	7.42±1.84	76.47±8.32**	17.65±4.8*	23.53±8.32*
BBM	46.88±14.88	1.56±3.13	1.56±3.13	49.26±12.09	2.86±3.3	2.14±1.29	56.29±11.63	8.42±3.12	11.19±6.32
PLT5-GRFs	68.89±13.61**	13.27±5.73**	14.65±7.29**	64.65±11.82*	23.57±9.67**	38.86±3.90**	73.49±10.24**	37.96±12.66**	64.21±22.17**
PLT5-IPT	59.74±5.14**	4.41±2.94*	4.41±2.94*	74.45±12.78**	5.97±4.81	23.32±10.44*	79.14±10.1**	22.7±14.3	34.83±23.11*
PLT5-WUS	52.08±7.98**	2.08±4.17	2.08±4.17	60.42±7.98**	4.17±4.81	3.32±3.55	68.75±10.49*	27.08±15.77	29.58±18.48
PLT5-WIND1	61.4±9.48**	7.54±3.31**	9.01±3.62**	58.46±12.03*	6.07±5.11	11.39±4.92*	61.31±10.81	19.49±6.05*	25.55±12.78
PLT5-ESR1	53.85±10.88**	3.85±7.69	3.85±7.69	55.77±3.85**	7.69±6.28	11.83±5.06*	63.46±3.85*	28.85±7.36**	42.31±16.01*
PLT5-WOX5	65.1±12.88**	1.56±3.13	1.56±3.13	66.67±10.62**	3.13±3.61	3.04±2.05	76.35±10.39**	22.19±7.93*	30.21±15.73
PLT5-WOX13	46.88±3.61**	1.56±3.13	1.56±3.13	59.38±8.07*	4.69±5.98	2.68±2.94	68.75±5.1**	10.94±5.98	12.5±8.84
PLT5-BBM	53.13±11.97*	1.56±3.13	1.56±3.13	58.02±14.32	5.64±4.54	9.10±1.64*	65.04±7.64*	10.32±6.17	13.36±8.25
GRFs-WOX5	19.64±6.84	1.79±3.57	1.79±3.57	39.36±14.65	5.73±7.86	4.43±4.98	53.87±6.8	12.65±7.64	16±9.41
GRFs-WOX13	31.25±8.84	3.13±6.25	3.13±6.25	33.82±10.05	4.41±5.63	9.72±4.07	42.65±8.82	8.82±3.4	16.18±7.4
P-GRFs-ESR1	31.94±6.99	11.11±4.54**	15.28±5.32**	45.83±8.33	9.72±5.32	13.58±5.40*	51.39±6.99	11.11±4.54	15.28±5.32
P-GRFs-WOX5	46.51±11.69*	4.6±5.95	4.6±5.95	52.3±10.34*	11.86±6.69	11.71±7.27	50.83±4.56	23.9±7.86*	29.78±8.0*
P-GRFs-WOX13	41.67±9.62	2.08±4.17	2.08±4.17	51.54±6.52*	10.51±4.52	14.20±4.97*	58.97±10.77	15.51±3.14*	22.44±8.33
P-WUX-WOX5	39.05±4.54	1.47±2.94	1.47±2.94	44.85±6.52	2.94±3.4	2.70±2.20	53.68±6.06	11.52±4.41	11.52±4.41
P-ESR1-WOX5	38.24±12.25	1.47±2.94	1.47±2.94	44.12±12.25	4.41±5.63	3.78±3.25	55.88±11.26	14.71±7.59	22.06±11.14
G-WOX5-ESR1	41.18±8.32	4.41±2.94*	4.41±2.94*	47.06±6.79	16.18±2.94**	16.52±7.77*	57.35±10.05	27.94±8.82**	41.18±9.61**
P-ESR1-WOX13	57.89±7.44**	3.95±2.63*	5.26±4.3	63.16±9.61**	15.79±6.08*	19.91±5.17*	71.05±6.79**	38.16±8.99**	53.95±11.67**

Note: Cotyledon explants (n = 20) were cultured on callus-induction MS medium supplemented with 2 mg/L zeatin, 0.15 mg/L indole-3-acetic acid (IAA), 100 mg/L kanamycin, and 100 mg/L Timentin for 2, 3, and 4 weeks to evaluate the effects of various DRs on plant regeneration and transformation. Callus induction frequency (%), shoot induction frequency (%), and transformation frequency (%) were calculated as described in Supplementary Table 1. Data are presented as mean ± s.d. from three independent experiments. Asterisks indicate statistically significant differences compared to the control (CK) based on a two-tailed Student's t-test (*P < 0.05; **P < 0.01).

Supplementary Table 3: Effects of developmental regulators (DRs) on plant regeneration and transformation in chili pepper (*Capsicum annuum*).

Developmental Regulator (DRs)	3 weeks post-inoculation		4 weeks post-inoculation	
	Shoot induction frequency (%)	Transformation frequency (%)	Shoot induction frequency (%)	Transformation frequency (%)
CTK	0	0	1.92±3.33	1.92±3.33
PLT5	1.92±3.33	1.92±3.33	3.71±3.71	3.71±3.71
GRFs	0	0	4.01±4.02	4.01±4.02
IPT	0	0	5.93±3.43	5.93±3.43
PLT5-GRFs	19.46±9.37*	21.52±7.62**	36.49±6.11**	56.09±12.57**
PLT5-IPT	7.69±5.44*	9.62±6.38*	13.46±6.38*	28.85±8.38**
PLT5-WUS	1.92±3.33	1.92±3.33	7.42±8.06	11.40±6.74
PLT5-WIND1	3.85±3.85	4.01±4.01	11.54±8.60	11.54±8.60*
PLT5-ESR1	7.58±5.07*	9.64±3.34*	9.36±7.70	9.78±8.35
PLT5-WOX5	4.01±4.01	4.01±4.01	9.78±8.35	9.78±8.35
PLT5-WOX13	3.85±3.85	5.63±6.34	7.42±5.06	9.48±6.42
PLT5-BBM	3.85±3.85	4.01±4.01	7.85±5.45	7.85±5.45
PLT5-GRFs+WOX13	9.48±8.42**	17.51±6.96**	23.17±9.64**	31.04±15.22**

Note: Cotyledon explants (n = 20) were cultured on callus-induction MS medium supplemented with 3 mg/L zeatin, 0.3 mg/L 1-naphthaleneacetic acid (NAA), 100 mg/L kanamycin, and 100 mg/L Timentin for 3 and 4 weeks to evaluate the effects of various DRs on plant regeneration and transformation. Shoot induction frequency (%) and transformation frequency (%) were calculated as described in Supplementary Table 1. Data are presented as mean ± s.d. from three independent experiments. Asterisks indicate statistically significant differences compared to the control (CK) based on a two-tailed Student's t-test (*P < 0.05; **P < 0.01).

Supplementary Table 4: Primers for vector construction, genotyping, and gene expression analysis.

Primer names	Primers (5'-3')	Accession No.	Purpose
AtPLT5-F-BsaI	aaGGTCTCATCGAATGAAGAACAATAACAACAAATCTTCTT	AT5G57390	Constructing pOX135-based vectors carrying various combinations of developmental regulator (DR) genes
AtPLT5-R-BsaI	AtGGTCTCGTGCATCATTCCAACCCAAAAACCG		
VisGRF4-F-BsaI	AaGGTCTCATCGAATGAAGCAGAGCTTTGTGG	LOC100259737 LOC100253609	
VisGIF1-R-BsaI	AtGGTCTCGTGCATCAATTCCCATCTTCAGCAG		
IPT-F-BsaI	ATGACAAATTGCTTTCAAGGA	DQ058764.1	
IPT-R-BsaI	TCACATTCGAAATGGTGG		
AtWUS1-F-BsaI	AaGGTCTCATCGAATGGAGCCGCCACAG	AT2G17950	
AtWUS1-R-BsaI	AtGGTCTCGTGCACTAGTTCAGACGTAGCTCAAGA		
AtWOX5-F-BsaI	AaGGTCTCATCGAATGTCTTTCTCCGTGAAAG	AT3G11260	
AtWOX5-R-BsaI	AtGGTCTCGTGCATTAAAGAAAGCTTAATCGAAGATC		
AtWOX13-F-BsaI	aaGGTCTCATCGAATGATGGAATGGGATAATCAG	AT4G35550	
AtWOX13-R-BsaI	AtGGTCTCGTGCATCAGCCTGACATGCC		
AtBBM-F-BsaI	aaGGTCTCATCGAATGAACTCGATGAATAACTGG	AT5G17430	
AtBBM-R-BsaI	atGGTCTCGTGCACTAAGTGTCGTTCCAAACTG		
AtWIND1-F-BsaI	aaGGTCTCATCGAATGGCAGCTGCTATGAA	AT1G78080	
AtWIND1-R-BsaI	atGGTCTCGTGCACTAAGCTAGAATCGAATCCC		
AtESR1-F-BsaI	aaGGTCTCATCGAATGGAAAAAGCCTTGAGAA	AT1G12980	
AtESR1-R-BsaI	atGGTCTCGTGCACTATCCCCACGATCTTCG		
MluI-35S-F	cgACGCGTATTGATGTGATAACATGGTGGAG	AB294426.1	
Hster-AatII-R	atGACGTCGGGCCTAGGGAGCT		
SpeI.35s-F	ccACTAGTATTGATGTGATAACATGGTGGAG	AB294426.1	
Hster.SalI-R	atGTCGACGGGCCTAGGGAGCT		
GFP_detector-F	ACAAGTTCAGCGTGTCCG	AB294426.1	Genotyping of the transgene in putative transgenic plants
GFP_detector-R	TCACCTTGATGCCGTTCT		
AmROSEA1-F	ATGGAAAAGAATTGTCGTGG	DQ275529.1	
AmROSEA1-R	TTAATTTCCAATTTGTTGGGC		
RUBY-F	TGGGTTCCACTCATGCTCAT	Addgene_160908	
RUBY-R	AGGAATGGTGGTGAAGGAGG		
CaGAPDH-qF	ATGATGATGTGAAAGCAGCG	LOC107848523	qRT-PCR analysis to assess the gene expression levels in transgenic chili pepper calli.
CaGAPDH-qR	TTTCAACTGGTGGCTGCTAC		
AtPLT5-qF	GCGTTGTCTTCTCTTCCGAC	AT5G57390	
AtPLT5-qR	ATGTACGCTAGAcACCTCCG		
VisGRF4-qF	CATTCGTTCTTCAGCAGCGA	LOC100259737	
VisGRF4-qR	AGCTGGGTTGTGGAGAATGA		