

## **Supplementary material**

### **Regulation of carotenoid biosynthesis and degradation in lettuce (*Lactuca sativa* L.) from seedlings to harvest**

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## **Supplementary Tables**

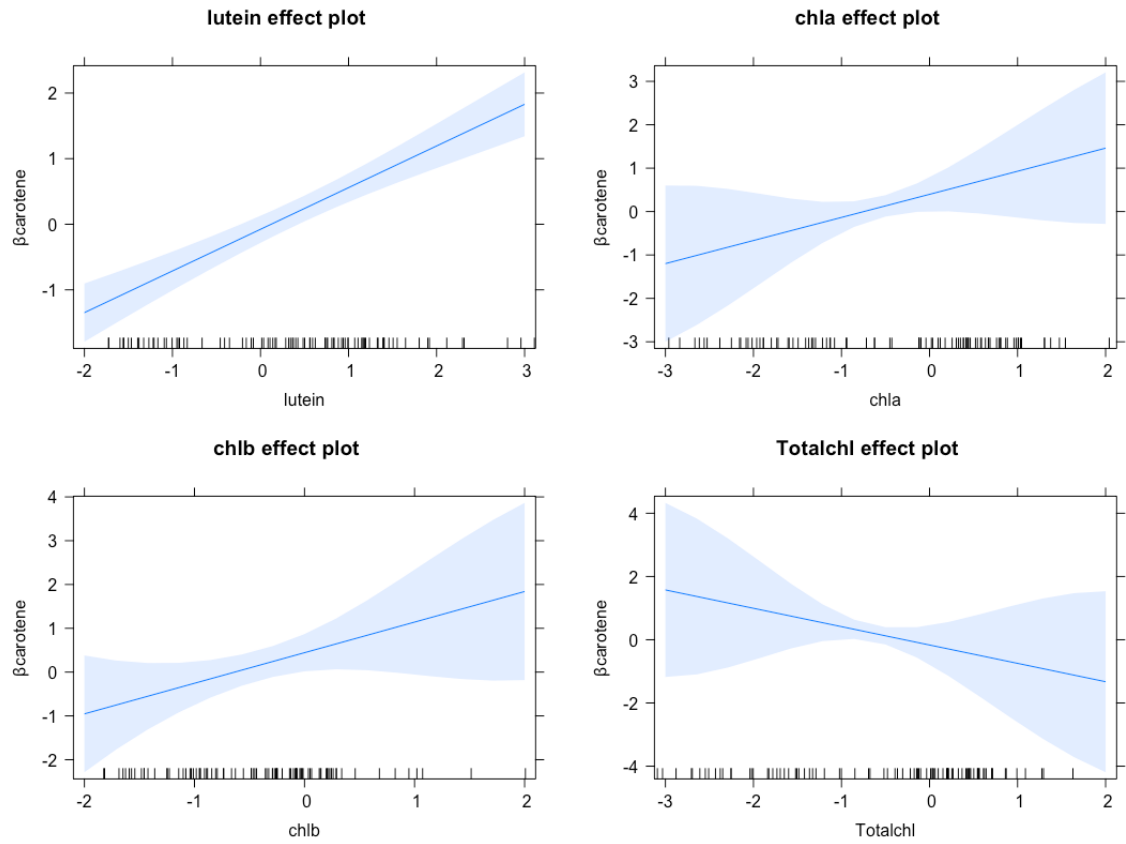
**Supplementary Table S1.** Primers sequences (designed by Quantprime) used for qRT-PCR analysis.

Locus	Foward primer	Reverse primer	Accession number
<i>UBQ</i>	5'-CCATTTGGTCCTCCGTCTCC-3'	5'-CGAGGGTTCTGCCGTCTTCT-3'	DW141651
<i>DXS</i>	5'-CGCCATTGATGACAGACCCAG-3'	5'-GCCCTTCCAGCATTATTCGC-3'	AB205044
<i>DXR</i>	5'-AGAAACGAATCTTTGGTTGAAG-3'	5'-TCACACAATCAGGATGACGG-3'	AB205045
<i>IPI</i>	5'-TCACTTGACGGAGAAGATTGAG-3'	5'-CCATACCAAAGGAAATGTCACC-3'	AB205048
<i>GGPS</i>	5'-AATTCGGTAAATCAAGCCCTAG-3'	5'-ATACACAGCATCGGTCGGAC-3'	AB205047
<i>GGPPS</i>	5'-AACGCCACCCACAATCCATGAAG-3'	5'-ATGCAGAGAATGGGCCTCACAC-3'	DY975567
<i>PSY1</i>	5'-ACGACATCGTACACCATCTGCTC-3'	5'-TTCCAGGGTTGTGGTGGCTAAC-3'	DY974614
<i>PSY2</i>	5'-GCTTGACGCTGCTTTATCAGAC-3'	5'-TCTTCAGATCCATCCTCATTCC-3'	AB205050
<i>PDS</i>	5'-TAGCTGATGCAGGTCACAAGCC-3'	5'-TTCCAAGCTGCCACCTTTCCAC-3'	DY972145
<i>ZDS</i>	5'-ATCCACCTCATGCCCTTGATCC-3'	5'-TATCATAGGTGCTGGCCTTGCTG-3'	DY960874
<i>CRTISO</i>	5'-ATCTGTGATGTTGCGATTGAGC-3'	5'-ACGGTGGTTGGATCGGGTATC-3'	AB205043
<i>LCY-B1</i>	5'-AGGAAGATCAGGGCCAATGAGTCC-3'	5'-TCTTGCTGCAGAGTCAGCCAAAC-3'	DY968060
<i>LCY-B2</i>	5'-AAGGGTCTCGTGGTGGATC-3'	5'-TGAGGTGAAGGGTCAATCG-3'	AB205041
<i>LCY-ε</i>	5'-TCACACATCGTGCTAATGGATC-3'	5'-CCGTTGAAGATAAAGAAGACCC-3'	AB205046
<i>CHY</i>	5'-TAACCGGAAGGAGCGTGAAGT-3'	5'-TTTGCCTGCACTCCTCTTGGAC-3'	DY970341
<i>CHYβ</i>	5'-TCCGAACGCTTTACTTACCTTG-3'	5'-GAACATCTCCACCCTCCATTG-3'	AB205042
<i>VDE</i>	5'-ACTCGCAACAATCGTCCTGAC-3'	5'-GGGCACACATTTCTTTCGG-3'	AB205051
<i>ZEP</i>	5'-GTTGGGTATCGGGTGTCTTGG-3'	5'-GGCTGGTTCGTTGTGAAATGC-3'	AB205053

**Supplementary Table S2.** Regression analysis of factors affecting  $\beta$ -carotene accumulation. **A.** without considering plant genotype, plant developmental stages or leaves position.

	$\beta$	SE	STD $\beta$	t	p	95.0% CI	df	F	p
(Constant)	-70.29	19.29		-3.64	0.00	-108.66, -31.93	4, 85	21.01	<0.001
Lutein	5.03	0.67	0.59	7.46	0.00	3.69, 6.37			
Chla	0.08	0.05	0.55	1.50	0.14	-0.03, 0.18			
Chlb	0.09	0.05	0.42	1.67	0.09	-0.02, 0.20			
ChlT	-0.05	0.05	-0.56	-1.03	0.31	-0.15, 0.05			

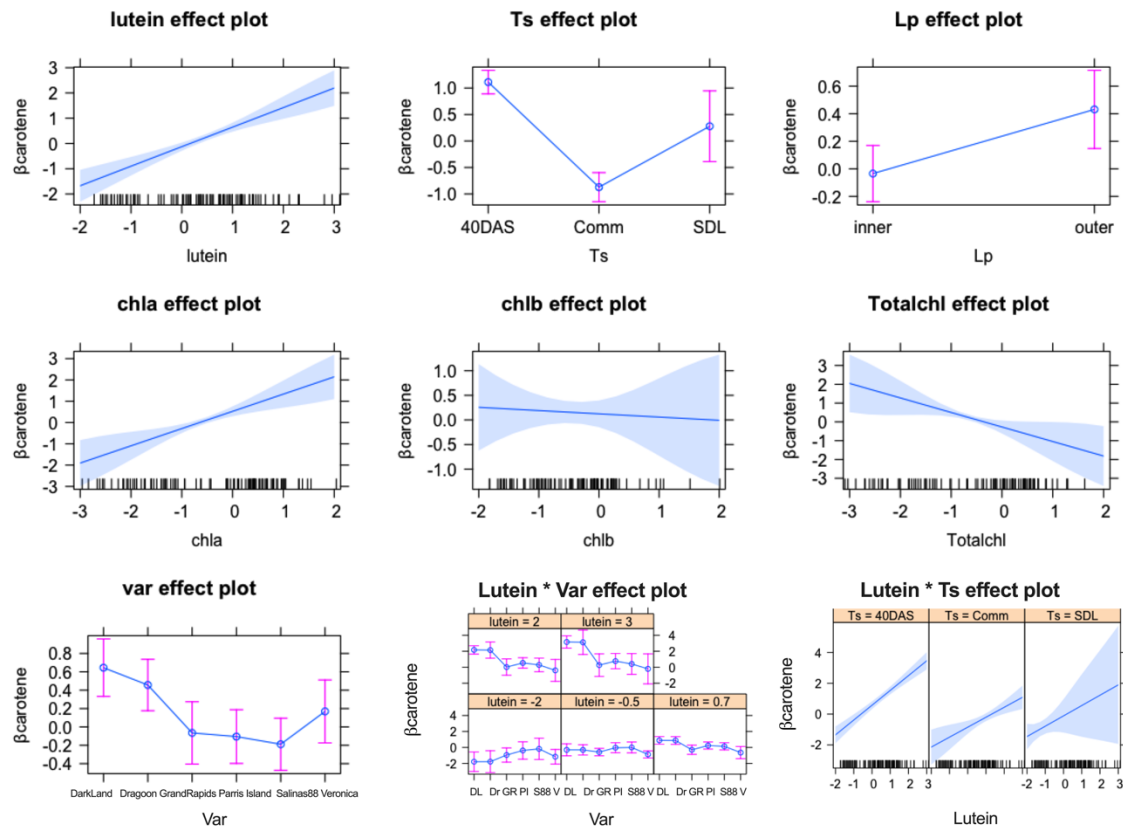
Model's AIC: 927.87; BIC: 942.87



**Supplementary Table S2.** Regression analysis of factors affecting  $\beta$ -carotene accumulation **B.** accounting role of genotype, sampling time and leave position.

	$\beta$	SE	STD $\beta$	t	p	95.0% CI	df	F	p
(Constant)	-68.29	18.85		-3.62	0.00	-105.79, -30.78	7, 89	39.65	<0.001
Variety	-4.53	1.93	-0.14	-2.34	0.02	-8.37, -0.68			
Sampling time (Ts)	-66.39	7.72	-0.89	-8.60	0.00	-81.74, -51.04			
Leaves position (Lp)	57.91	8.44	0.51	6.86	0.00	41.11, 74.70			
Lutein	10.42	0.84	1.22	12.40	0.00	8.74, 12.09			
Chla	0.04	0.04	0.29	1.12	0.27	-0.03, 0.11			
Chlb	0.10	0.04	0.49	2.74	0.01	0.03, 0.18			
ChIT	-0.07	0.04	-0.78	-2.07	0.04	-0.15, -0.01			

Model's AIC: 644.06; BIC: 719.06



**Supplementary Table S3.** Pearson's correlation (r) comparing relative gene expression with total chlorophyll, lutein and  $\beta$ -carotene in inner and outer lettuce leaves at seedling stage, 40 days after sowing (DAS) stage and commercial size stage.

Gene	Comm <sup>1</sup>	Seedling			40 DAS inner			40 DAS outer			Commercial inner			Commercial outer		
		Chl T	Lut <sup>2</sup>	$\beta$ -car <sup>3</sup>	Chl T	Lut	$\beta$ -car	Chl T	Lut	$\beta$ -car	Chl T	Lut	$\beta$ -car	Chl T	Lut	$\beta$ -car
<i>DXS</i>	Inner	0.56	0.64	0.41	0.11	0.69	0.5	-0.61	0.01	-0.2	-0.85	-0.31	-0.4	-0.90**	-0.44	-0.51
	Outer	0.21	0.43	0.07	-0.1	0.66	0.29	-0.23	0.12	0.09	-0.35	-0.21	-0.08	-0.55	-0.37	-0.29
<i>DXR</i>	Inner	-0.17	-0.4	-0.55	-0.39	-0.68	-0.83	-0.67	-0.39	- 0.66	-0.26	-0.16	-0.4	0.1	-0.19	0.13
	Outer	0.23	-0.02	0.15	-0.06	-0.4	-0.22	-0.3	-0.1	-0.1	0.6	0.11	0.45	-0.09	-0.14	0
<i>IPI</i>	Inner	0.63	0.02	0.17	-0.04	-0.64	-0.5	0.92**	0.65	0.55	-0.23	0.06	-0.12	-0.43	-0.68	-0.47
	Outer	-0.11	-0.2	-0.4	-0.04	-0.54	-0.33	0.54	0.61	0.36	0.51	0.49	0.73	0.12	-0.46	0.05
<i>GGPS</i>	Inner	-0.33	-0.36	-0.33	-0.48	-0.87	-0.73	-0.48	-0.91	- 0.78	-0.67	-0.25	-0.25	-0.54	-0.35	-0.44
	Outer	-0.9	-0.36	-0.67	-0.67	-0.9	-0.83	-0.14	-0.77	-0.4	0.09	-0.19	0.17	-0.27	0.03	0.09
<i>GGPPS</i>	Inner	0.53	0.43	0.25	0.37	0.73	0.69	-0.46	-0.18	- 0.46	-0.34	-0.4	-0.35	-0.15	-0.11	-0.45
	Outer	0.92**	0.59	0.72	0.71	0.7	0.79*	0.25	0.22	0.4	0.57	-0.24	0.34	-0.29	0	-0.29
<i>PSY1</i>	Inner	0.71	0.72	0.65	0.82*	0.85*	0.89**	0.07	-0.08	-0.2	0.37	0.43	0.18	0.66	0.7	0.85
	Outer	0.92**	0.78*	0.84*	0.74	0.77	0.73	0.61	0.3	0.57	0.85*	0.54	0.65	0.54	0.72	0.71
<i>PSY2</i>	Inner	0.5	0.34	0.34	0.66	0.16	0.37	-0.66	-0.66	- 0.76	-0.28	0.27	0.29	-0.04	-0.4	-0.4
	Outer	-0.42	0.23	-0.27	0.15	0.1	0.03	0.23	-0.43	0.04	0.33	0.49	0.73	0.52	-0.1	0.3
<i>PDS</i>	Inner	-0.41	-0.72	-0.77	-0.11	-0.54	-0.19	-0.13	-0.47	-0.5	-0.11	-0.55	-0.49	0.5	0.02	-0.1
	Outer	0.15	-0.4	-0.04	-0.03	-0.59	-0.24	0.22	-0.12	0.12	0.45	-0.34	0.12	0.49	-0.05	-0.03
<i>ZDS</i>	Inner	-0.1	-0.46	-0.47	-0.52	-0.32	-0.4	0	-0.35	- 0.43	0.05	-0.3	-0.14	0.64	0.41	0.4
	Outer	0.38	-0.13	0.22	-0.43	-0.52	-0.54	0.28	-0.01	0.16	0.59	-0.3	0.19	0.29	0.56	0.38
<i>CRTISO</i>	Inner	0.47	0.39	0.46	-0.32	-0.41	-0.56	-0.63	-0.81	- 0.88	0.7	0.82*	0.79*	-0.93**	-0.92**	-0.86*

	Outer	-0.13	0.45	0.14	-0.75	-0.38	-0.7	-0.15	-0.48	- 0.21	0.81*	0.76*	0.76*	-0.35	-0.76*	-0.37
<i>LCY-β1</i>	Inner	0.42	0.39	0.26	0.32	0.26	0.13	-0.16	0.04	- 0.24	0.36	0.35	0.03	0.01	0.21	0.08
	Outer	0.98***	0.53	0.77*	0.96***	0.46	0.76*	0.39	0.45	0.54	0.53	0.36	0.27	0.56	0.62	0.80*
<i>LCY-β2</i>	Inner	0.73	0.7	0.65	-0.42	-0.44	-0.72	-0.58	-0.8	- 0.93	0.77*	0.85*	0.91**	0.03	-0.23	-0.11
	Outer	-0.16	0.52	-0.03	-0.25	-0.41	-0.46	-0.39	-0.76	- 0.66	0.55	0.63	0.5	0.24	0.08	0.31
<i>LCY-ε</i>	Inner	0.72	0.54	0.39	0.4	0.01	0.19	-0.67	-0.45	- 0.69	-0.42	-0.12	-0.42	-0.31	-0.73	-0.69
	Outer	-0.05	0.42	-0.09	-0.08	0.08	-0.02	-0.4	-0.16	-0.2	0.37	0.2	0.41	-0.39	-0.56	-0.49
<i>CHY</i>	Inner	-0.1	-0.32	-0.37	0.23	-0.12	0.21	-0.15	-0.37	- 0.62	-0.31	-0.21	-0.51	0.29	0.08	0.12
	Outer	0.66	-0.2	0.26	-0.09	-0.45	-0.36	0.01	0.45	0.3	0.18	-0.14	-0.05	-0.36	0.15	-0.17
<i>CHY-β</i>	Inner	0.68	0.23	0.08	0.31	0.01	-0.16	-0.75	-0.71	- 0.78	-0.22	0.07	-0.28	-0.36	-0.47	-0.28
	Outer	0.22	0.16	-0.1	0.75	0.18	0.39	-0.27	-0.72	- 0.47	0.41	0.29	0.39	-0.12	-0.2	0.08
<i>VDE</i>	Inner	0.51	0.11	0.17	0.27	-0.27	-0.09	-0.79	-0.57	- 0.81	0.08	0.49	0.56	-0.2	-0.41	-0.55
	Outer	-0.2	0.11	-0.19	0.05	-0.15	-0.07	-0.22	-0.36	- 0.21	0.59	0.63	0.86*	0.35	-0.03	0.25
<i>ZEP</i>	Inner	0.56	0.42	0.58	0.54	0.12	0.19	0.09	0.28	0	-0.02	0.11	-0.16	-0.16	-0.48	-0.26
	Outer	0.42	0.55	0.57	0.7	0.27	0.49	0.66	0.63	0.74	0.67	0.25	0.46	-0.08	-0.26	-0.03

<sup>1</sup> Commercial size; <sup>2</sup> Lutein; <sup>3</sup> β-carotene; \* Significant ( $P < 0.05$ ); \*\* Significant ( $P < 0.01$ ); \*\*\* Significant ( $P < 0.001$ ).

**Supplementary Table S4.** An expression correlation analysis using *LsLCY-β1* & *LCY-β2* as the driver genes for β-carotene biosynthesis with all other carotenoid biosynthesis pathway genes.

Control Variables	Gene	DXS	DXR	IPI	GGPS	GGPPS	PSY1	PSY2	PDS	ZDS	CRTISO	LCY-ε	CHYε	CHYβ	VDE	ZEP
LCY-β1 & LCY-β2	DXS	1	-0.296	0.276	0.269	0.147	0.053	0.331	0.134	0.166	0.217	0.520**	-0.097	0.865**	0.367	0.221
	DXR	-0.296	1	-0.15	0.023	-0.08	-0.448*	0.141	-0.237	-0.322	0.103	-0.079	0.075	-0.259	0.433*	0.37
	IPI	0.276	-0.15	1	0.307	0.188	0.419*	0.706**	0.581**	0.447*	0.740**	0.113	0.032	0.136	0.397*	0.495**
	GGPS	0.269	0.023	0.307	1	0.248	0.102	0.708**	0.336	0.104	0.318	0.570**	0.011	0.508**	0.334	0.166
	GGPPS	0.147	-0.08	0.188	0.248	1	0.422*	0.077	0.367	0.269	0.164	0.169	0.325	0.081	-0.085	0.071
	PSY1	0.053	-0.448*	0.419*	0.102	0.422*	1	0.141	0.774**	0.773**	0.167	0.048	0.587**	0.051	-0.261	-0.129
	PSY2	0.331	0.141	0.708**	0.708**	0.077	0.141	1	0.451*	0.181	0.686**	0.381*	-0.104	0.460*	0.563**	0.385*
	PDS	0.134	-0.237	0.581**	0.336	0.367	0.774**	0.451*	1	0.794**	0.445*	0.284	0.549**	0.174	0.092	0.141
	ZDS	0.166	-0.322	0.447*	0.104	0.269	0.773**	0.181	0.794**	1	0.189	0.062	0.601**	0.146	-0.039	0.025
	CRTISO	0.217	0.103	0.740**	0.318	0.164	0.167	0.666**	0.445*	0.189	1	0.382*	-0.153	0.103	0.607**	0.794**
	LCY-ε	0.520**	-0.079	0.113	0.570**	0.169	0.048	0.381*	0.284	0.062	0.382*	1	0.02	0.532**	0.306	0.312
	CHYε	-0.097	0.075	0.032	0.011	0.325	0.587**	-0.104	0.549**	0.601**	-0.153	0.02	1	-0.081	-0.12	-0.043
	CHYβ	0.865**	-0.259	0.136	0.508**	0.081	0.051	0.460*	0.174	0.146	0.103	0.532**	-0.081	1	0.316	-0.032
	VDE	0.367	0.433*	0.397*	0.334	-0.085	-0.261	0.563**	0.092	-0.039	0.607**	0.306	-0.12	0.316	1	0.776**
	ZEP	0.221	0.37	0.495**	0.166	0.071	-0.129	0.385*	0.141	0.025	0.794**	0.312	-0.043	-0.032	0.776**	1

Note. Pink colour means Pearson correlation is >0.75. \*\*. Correlation is significant at 0.01 level.

\*. Correlation is significant at 0.05 level.



**Supplementary Table S5.** An expression correlation analysis using *CHYε* as the driver gene for lutein biosynthesis to determine the level of co-expression that *CHYε* shares with all of the other carotenoid biosynthesis pathway genes.

Control Variables	Genes	DXS	DXR	IPI	GGPS	GGPPS	PSY1	PSY2	PDS	ZDS	CRTISO	LCY-β1	LCY-β2	LCY-ε	CHYβ	VDE	ZEP
CHYε	DXS	1	0.169	0.502**	0.440*	-0.026	-0.164	0.583**	0.356	0.238	0.487**	0.776**	0.614**	0.868**	0.970**	0.578**	0.567**
	DXR	0.169	1	-0.014	0.089	-0.169	-0.643**	0.195	-0.221	-0.399*	0.143	0.342	0.14	0.221	0.171	0.371*	0.327
	IPI	0.502**	-0.014	1	0.545**	0.188	0.303	0.811**	0.756**	0.587**	0.816**	0.2	0.584**	0.544**	0.470*	0.660**	0.710**
	GGPS	0.440*	0.089	0.545**	1	0.269	0.031	0.818**	0.565**	0.288	0.638**	0.106	0.596**	0.648**	0.556**	0.634**	0.559**
	GGPPS	-0.026	-0.169	0.188	0.269	1	0.33	0.148	0.26	0.151	0.234	-0.23	0.1	0.035	-0.041	0.049	0.126
	PSY1	-0.164	-0.643**	0.303	0.031	0.33	1	0.047	0.518**	0.580**	0.105	-0.256	-0.116	-0.179	-0.162	-0.233	-0.172
	PSY2	0.583**	0.195	0.811**	0.818**	0.148	0.047	1	0.703**	0.429*	0.883**	0.192	0.813**	0.757**	0.652**	0.837**	0.788**
	PDS	0.356	-0.221	0.756**	0.565**	0.26	0.518**	0.703**	1	0.731**	0.715**	0.072	0.481**	0.487**	0.394*	0.480**	0.489**
	ZDS	0.238	-0.399*	0.587**	0.288	0.151	0.580**	0.429*	0.731**	1	0.464*	-0.042	0.321	0.237	0.243	0.275	0.292
	CRTISO	0.487**	0.143	0.816**	0.638**	0.234	0.105	0.883**	0.715**	0.464*	1	0.092	0.819**	0.716**	0.494**	0.850**	0.924**
	LCY-β1	0.776**	0.342	0.2	0.106	-0.23	-0.256	0.192	0.072	-0.042	0.092	1	0.238	0.604**	0.738**	0.183	0.212
	LCY-β2	0.614**	0.14	0.584**	0.596**	0.1	-0.116	0.813**	0.481**	0.321	0.819**	0.238	1	0.829**	0.651**	0.779**	0.808**
	LCY-ε	0.868**	0.221	0.544**	0.648**	0.035	-0.179	0.757**	0.487**	0.237	0.716**	0.604**	0.829**	1	0.883**	0.717**	0.747**
	CHYβ	0.970**	0.171	0.470*	0.556**	-0.041	-0.162	0.652**	0.394*	0.243	0.494**	0.738**	0.651**	0.883**	1	0.596**	0.528**
	VDE	0.578**	0.371*	0.660**	0.634**	0.049	-0.233	0.837**	0.480**	0.275	0.850**	0.183	0.779**	0.717**	0.596**	1	0.901**
	ZEP	0.567**	0.327	0.710**	0.559**	0.126	-0.172	0.788**	0.489**	0.292	0.924**	0.212	0.808**	0.747**	0.528**	0.901**	1

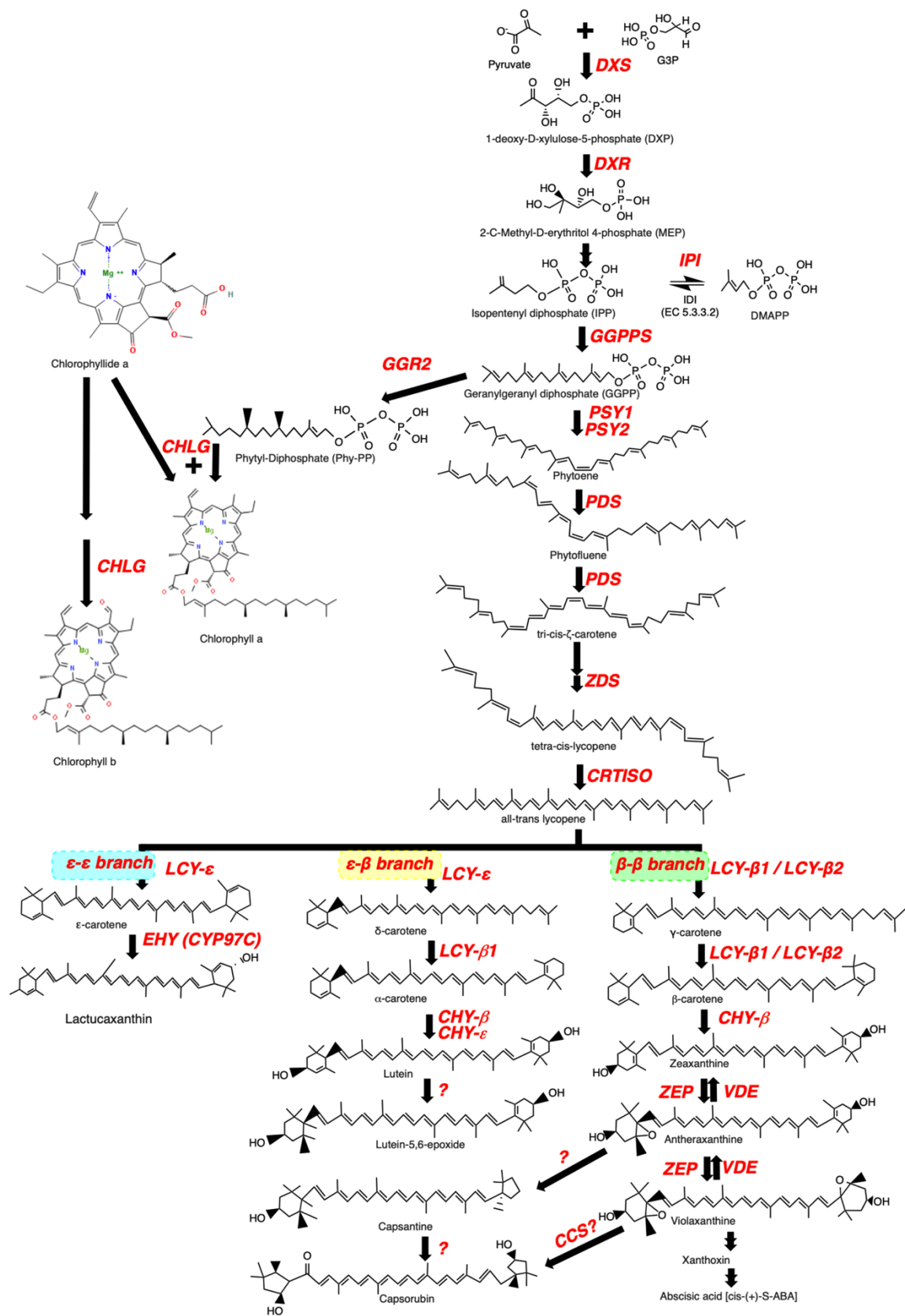
Note: Pink colour means Pearson correlation is >0.75. \*\*. Correlation is significant at 0.01 level.

\*. Correlation is significant at 0.05 level.

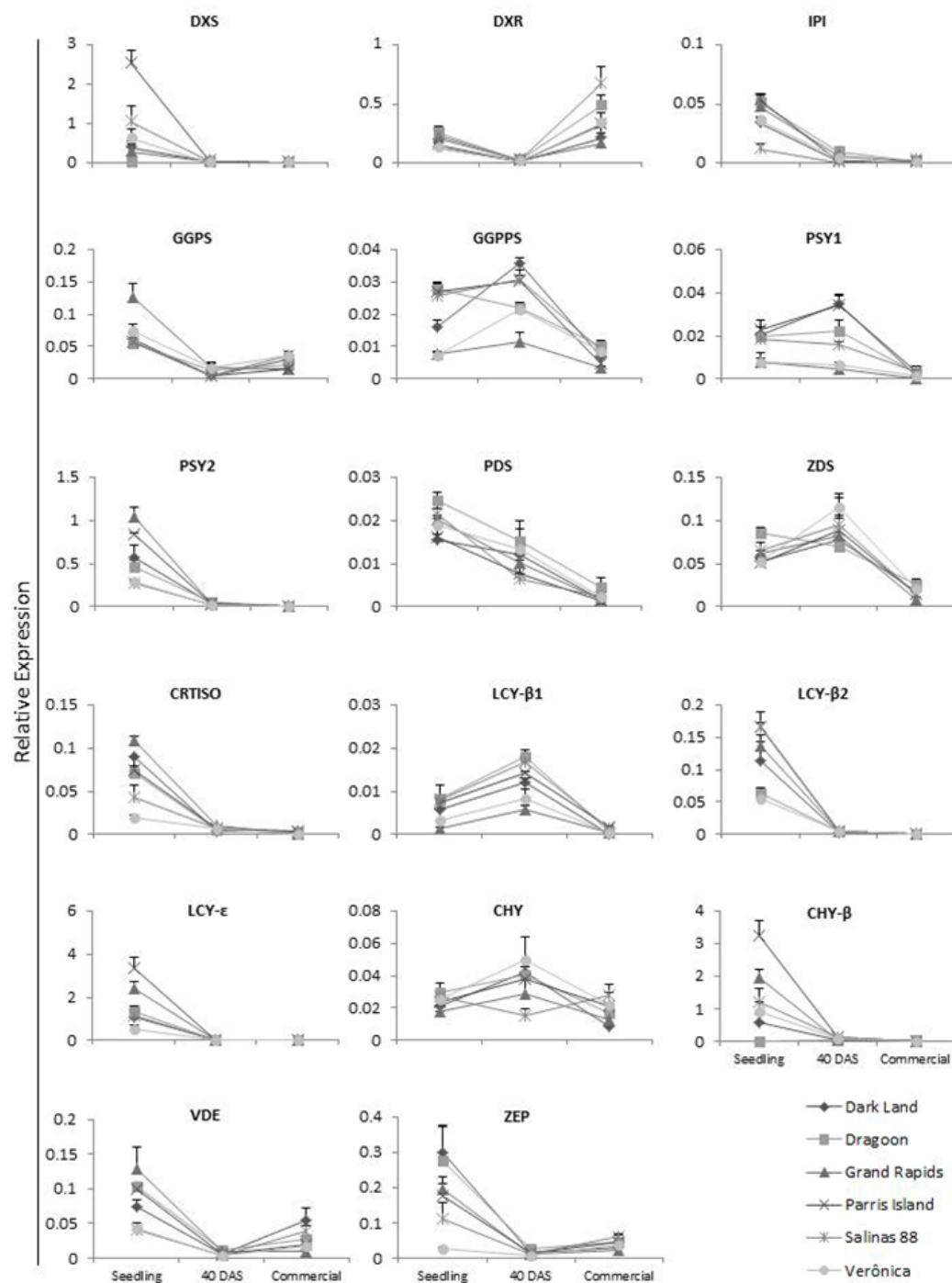
**Supplementary Table S6.** Lettuce plants used in the experiments.

<b>Genotype</b>	<b>Type</b>	<b>Genotype origin</b>	<b>Seeds origin</b>
DarkLand	Romaine	USA	UFLA Germplasm Bank
Dragoon	Mini Romaine	USA	UFLA Germplasm Bank
Grand Rapids	Loose leaf	USA	UFLA Germplasm Bank
Parris Island	Romaine	USA	UFLA Germplasm Bank
Salinas 88	Crisp Head	USA	UFLA Germplasm Bank
Verônica	Loose Leaf	Brazil	UFLA Germplasm Bank

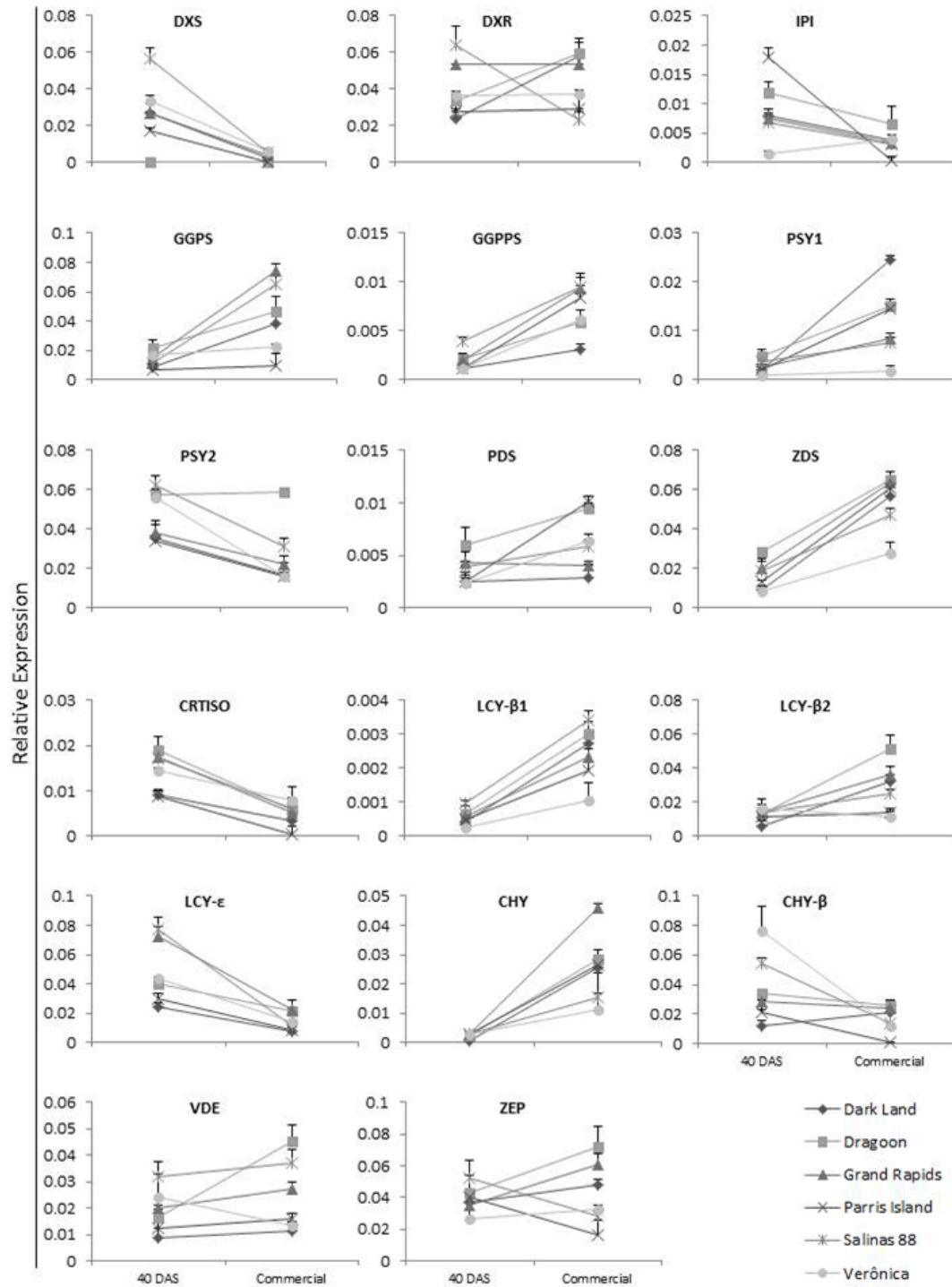
## Supplementary Figures



**Supplementary Figure S1.** Schematic representation of the non-mevalonate (methylerythritol 4-phosphate, MEP) chloroplasts pathway and mevalonate (cytosolic) pathway of carotenoid biosynthesis in *Lactuca sativa*, *Asteraceae*. The enzymes' names are highlighted in bold. Abbreviations: GA3P, glyceraldehyde-3-phosphate; DXS, 1-deoxyxylulose 5-phosphate synthase; DOXP, D-1-deoxyxylulose-5-phosphate; DXR, 1-deoxyxylulose 5-phosphate reductoisomerase; MEP, 2-C-methyl-D-erythritol-2,4- cyclodiphosphate; IPP, isopentenyl diphosphate; IPI, IPP isomerase; GGPP, geranylgeranyl diphosphate; GGPS, GGPP synthase; PSY1, phytoene synthase; PDS, phytoene desaturase; ZDS,  $\zeta$ -carotene desaturase; CRTISO, carotenoid isomerase; LCY-B, lycopene  $\beta$ -cyclase; LCY-E, lycopene  $\epsilon$ -cyclase; CHYB,  $\beta$ -ring hydroxylase; CHYE,  $\epsilon$ -ring hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin deepoxidase; EHY,  $\epsilon$ -carotene hydroxylase; CHLG, Chlorophyll synthase; GGR2, geranylgeranyl reductase2.



**Supplementary Figure S2.** Relative expression of lettuce carotenoid biosynthetic genes in inner leaves of different lettuce cultivar measured at seedling stage, 40 days after sowing (DAS), and commercial size (60 DAS) stages. Gene expression was measured relative to *UBIQUITIN*. Error bars are standard deviation of the mean from three biological replicates.



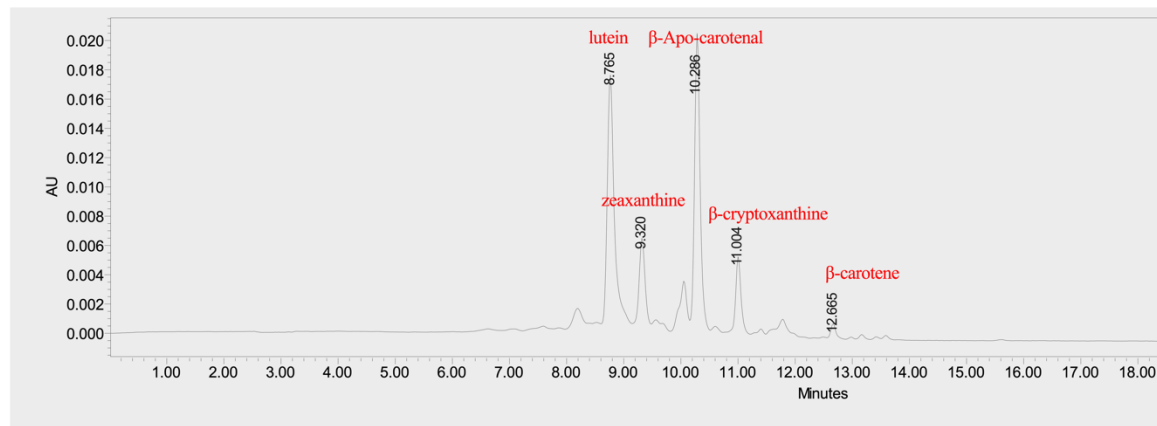
**Supplementary Figure S3.** Relative expression of lettuce carotenoid biosynthetic genes in outer leaves of different lettuce cultivar measured at 40 days after sowing (DAS) and commercial size (60 DAS) stages. Gene expression was measured relative to *UBIQUITIN*. Error bars are standard deviation of the mean from three biological replicates.



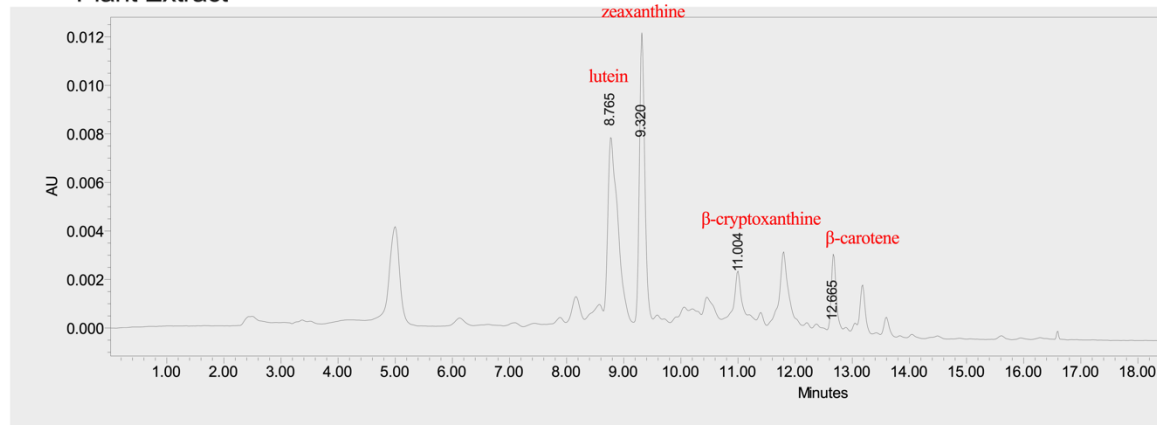
**Supplementary Figure S4.** Pearson correlation analysis of Relative expression of lettuce carotenoid biosynthetic genes in outer leaves of different lettuce cultivar measured 20days after sowing (SDL), at 40days after sowing (DAS) and commercial size (60DAS) stages. Only significant correlation is presented with Pearson correlation results. Correlation is significant at 0.05 level.



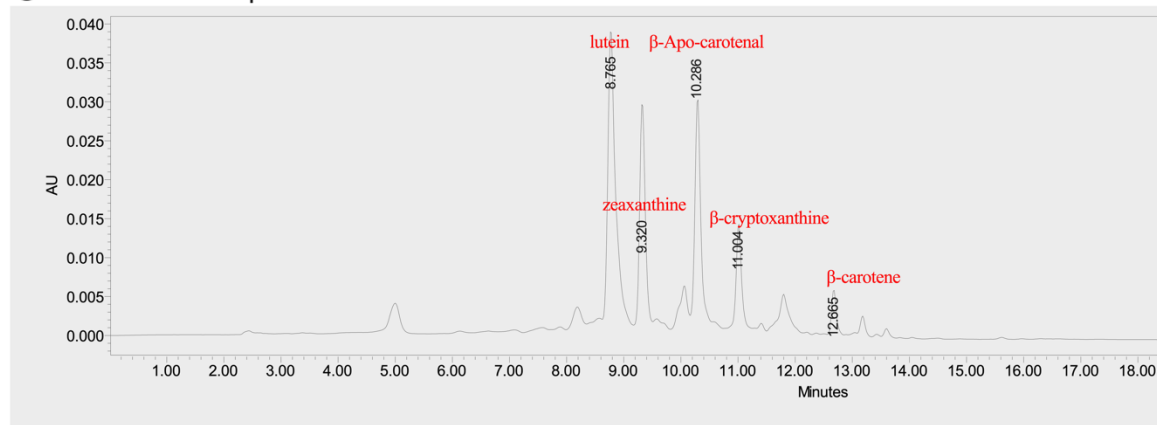
### A Carotenoids standards



### B Plant Extract



### C Plant Extract spiked with Carotenoids standards



**Supplementary Figure S5.** Carotenoids HPLC profile. A. Standards used to analyse carotenoids content in leaves. B. Plant extracts carotenoid profile. C. Plant extract spiked with carotenoids standards.