Supplementary Material

Comparative analysis of the plastid conversion, photochemical activity and chlorophyll degradation in developing embryos of green-seeded and yellow-seeded pea (*Pisum sativum* L.) cultivars

Galina Smolikova[A,H], *Olga Shiroglazova*[A], *Galina Vinogradova*[B], *Irina Leppyanen*[C], *Ekaterina Dinastiya*[D,E,F], *Olga Yakovleva*[G], *Elena Dolgikh*[C], *Galina Titova*[B], *Andrej Frolov*[D,F] *and Sergei Medvedev*[A]

[A]Department of Plant Physiology and Biochemistry, Saint Petersburg State University, Saint Petersburg, Russian Federation.

[B]Laboratory of Embryology and Reproductive Biology, Komarov Botanical Institute, Russian Academy of Sciences, Saint Petersburg, Russian Federation.

[C]Laboratory of Signal Regulation, All-Russia Research Institute for Agricultural Microbiology, Saint Petersburg, Russian Federation.

[D]Department of Biochemistry, Saint Petersburg State University, Saint Petersburg, Russian Federation.

[E]Postovsky Institute of Organic Synthesis, Ural Branch of Russian Academy of Sciences, Ekaterinburg, Russian Federation.

[F]Department of Bioorganic Chemistry, Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany.

[G]Laboratory of Anatomy and Morphology, Komarov Botanical Institute, Russian Academy of Sciences, Saint Petersburg, Russian Federation.

[H]Corresponding author. Email: g.smolikova@spbu.ru

Table S1 Primers to identify the full-size coding sequences of the chlorophyll degradation genes.

Primer pairs were designed using Vector NTI program and produced by Evrogen company (Moscow, Russia). ***NYC1*** (*NON-YELLOW COLORING 1)* encodes chlorophyll *b* reductase, converting chlorophyll *b* to 7-hydroxymethyl chlorophyll *a*; ***HCAR*** encodes 7-hydroxymethyl chlorophyll *a* reductase, converting 7-hydroxymethyl chlorophyll *a* to chlorophyll *a*; ***SGR*** genes (*STAY-GREEN*) encodes magnesium (Mg)-dechelatase, converting chlorophyll *a* to pheophytin *a; **PPH*** encodes pheophytin pheophorbide hydrolase/pheophytinase, converting pheophytin *a* to pheophorbide *a; **PAO*** encodes pheophorbide *a* oxygenase, which catalyzes oxidative ring opening in pheophorbide *a* and convert it to red chlorophyll catabolite (RCC); ***RCCR*** encodes red chlorophyll catabolite reductase that reduces RCC to FCC (fluorescent red chlorophyll catabolite)

Gene	Flanking primers	Internal PCR primers
NYC1	F 5`-TTACAAAGACATGTACCAATAAATAAACC-3`	F 5`-CTAATTGGGTTTTGGATGATTATTGTC-3`
	R 5`-ACATGAATTGCATGCCTCTCC-3`	R 5`-TTTCCTCAAGCTCTTTGACAGTTG-3`
HCAR	F 5`-GCCACATCAGTTTTCACTCACTG-3`	F 5`-GACGTTATTGCTCCATCTTGCTACAG-3`
	R 5`-CATTTCTCACTGCCATAGTTCATATC-3`	R 5`-GGAGCAGGCTGAGAAGGACC-3`
PAO	F 5`-GACTCAAACAATTCTTGTTTCTTTCC-3`	F1 5`-TTTCCTCAAGCTCTTTGACAGTTG-3`
	R 5`GTTAGTCCTCAACTCATATCATTTA-3`	R1 5`-GCTTTCTCCCAACCATTCTCATC-3`
		F2 5`-GGTAAGACTCGCTCCATTGTTTG-3`
		R2 5`-ATTTCTAAATGCCAACACAAAACG-3`
PPH	F 5`-ATGGAAACTCTTTCATATGGTTCTG-3`	
	R 5`-TTATATTTGATGGTGGCATAGTTCAC-3`	
RCCR	F 5`-CCCTACACGCACCACTTTCC-3`	F 5`-GAGGATCATTAGATATAACATCTATTTCAGG-3`
	R5`-TTTCTATCTTAGACAGTAAAATACTTC-3`	R 5`-GGTTGAACCTCATGAACTTTTTCC-3`

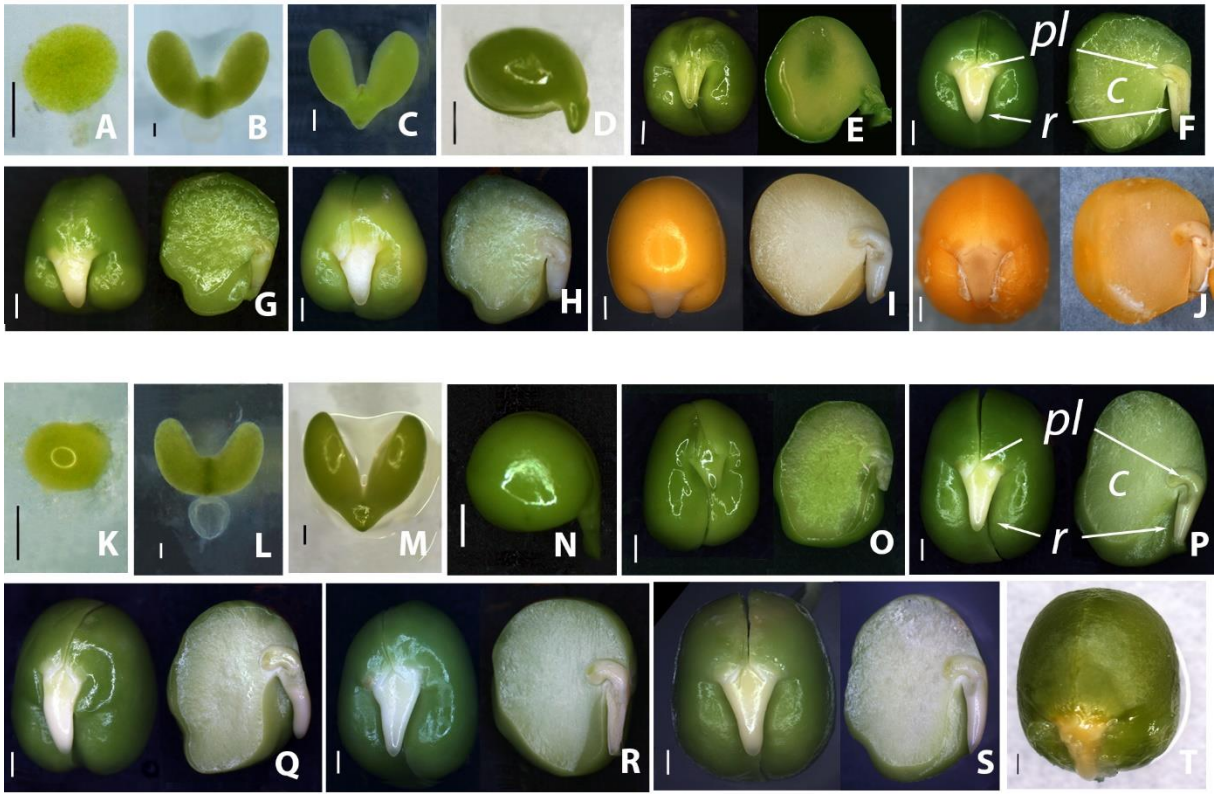

Figure S1 Images of developing pea embryos probed by light stereomicroscope Stemi 2000-C (CarlZeiss, Germany).

Frisson (yellow-seeded cultivar): **A** – 6th DAP (days after pollination), **B** – 7th DAP, **C** – 10th DAP, **D** – 14th DAP, **E** – 16th DAP (hereinafter: left - outside view, right – longitudinal sections), **F** – 18th DAP, **G** – 21th DAP, **H** - 24th DAP, **I** - 35th DAP, **J** – mature seeds.

Rondo (green-seeded cultivar): **K** – 6th DAP, **L** – 7th DAP, **M** – 10th DAP, **N** – 14th DAP, **O** – 16th DAP (hereinafter: left - outside view, right – longitudinal sections), **P** – 21th DAP, **Q** – 24th DAP, **R** - 35th DAP, **S** - 40th DAP, **T** – mature seeds.

c – cotyledons, ***pl*** – plumule, ***r*** – radicle.

Scale: 1.2 mm.

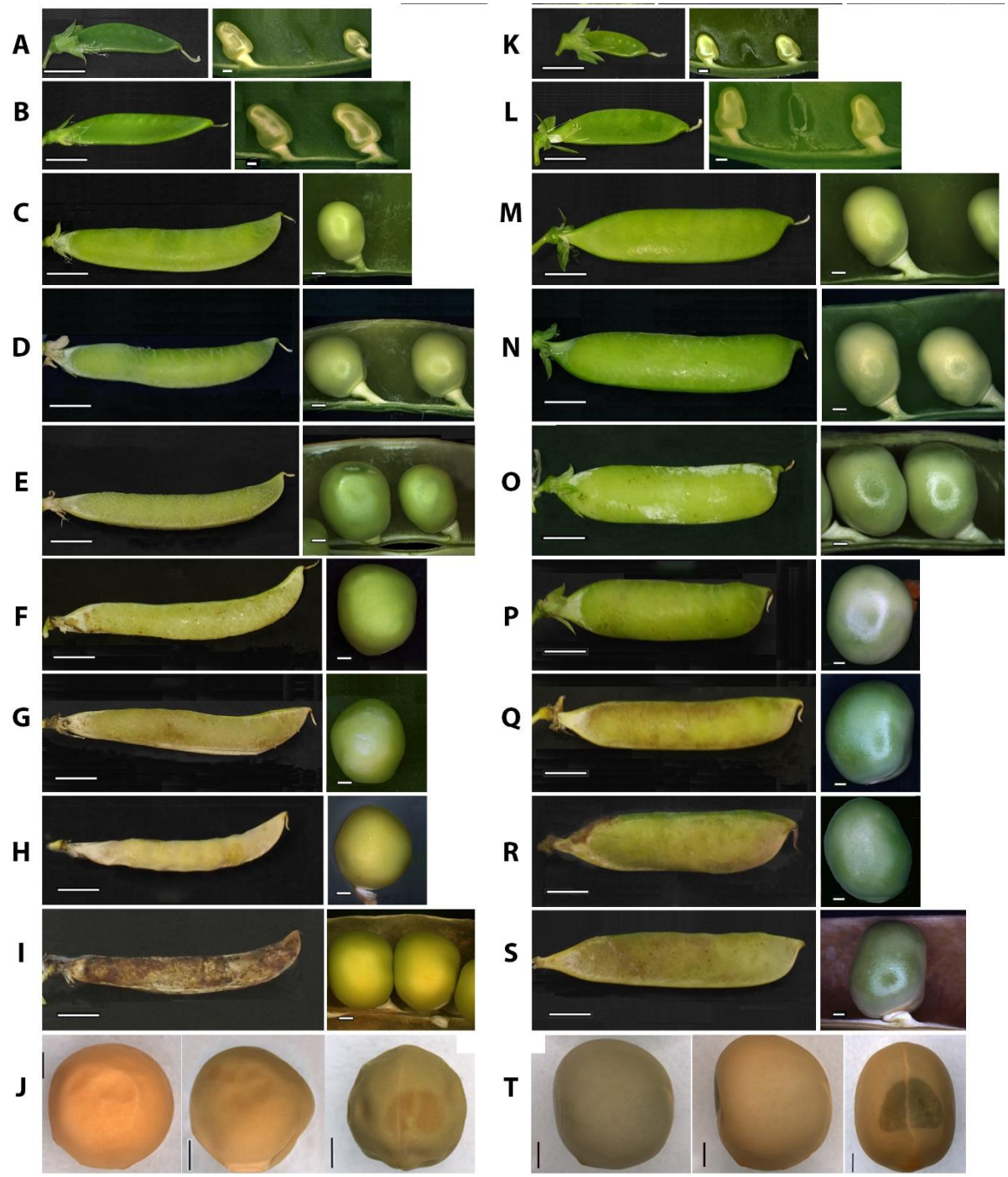

Figure S2 Images of developing pea pods and seeds probed by light stereomicroscope Stemi 2000-C (CarlZeiss, Germany).

Frisson (yellow-seeded cultivar): **A** – 2th DAP (hereinafter: left – pod, right - seeds), **B** – 4th DAP, **C** – 14th DAP, **D** – 16th DAP, **E** – 18th DAP, **F** – 21th DAP, **G** – 24th DAP, **H** - 35th DAP, **I** - 40th DAP, **J** – mature seeds (with different color of coats).

Rondo (green-seeded cultivar): **K** – 2th DAP (hereinafter: left – pod, right - seeds), **L** – 4th DAP, **M** – 14th DAP, **N** – 16th DAP, **O** – 21th DAP, **P** – 24th DAP, **Q** – 35th DAP, **R** - 40th DAP, **S** - 55th DAP, **T** – mature seeds (with different color of coats).

Scale: pods – 1.2 sm, seeds– 1.2 mm.

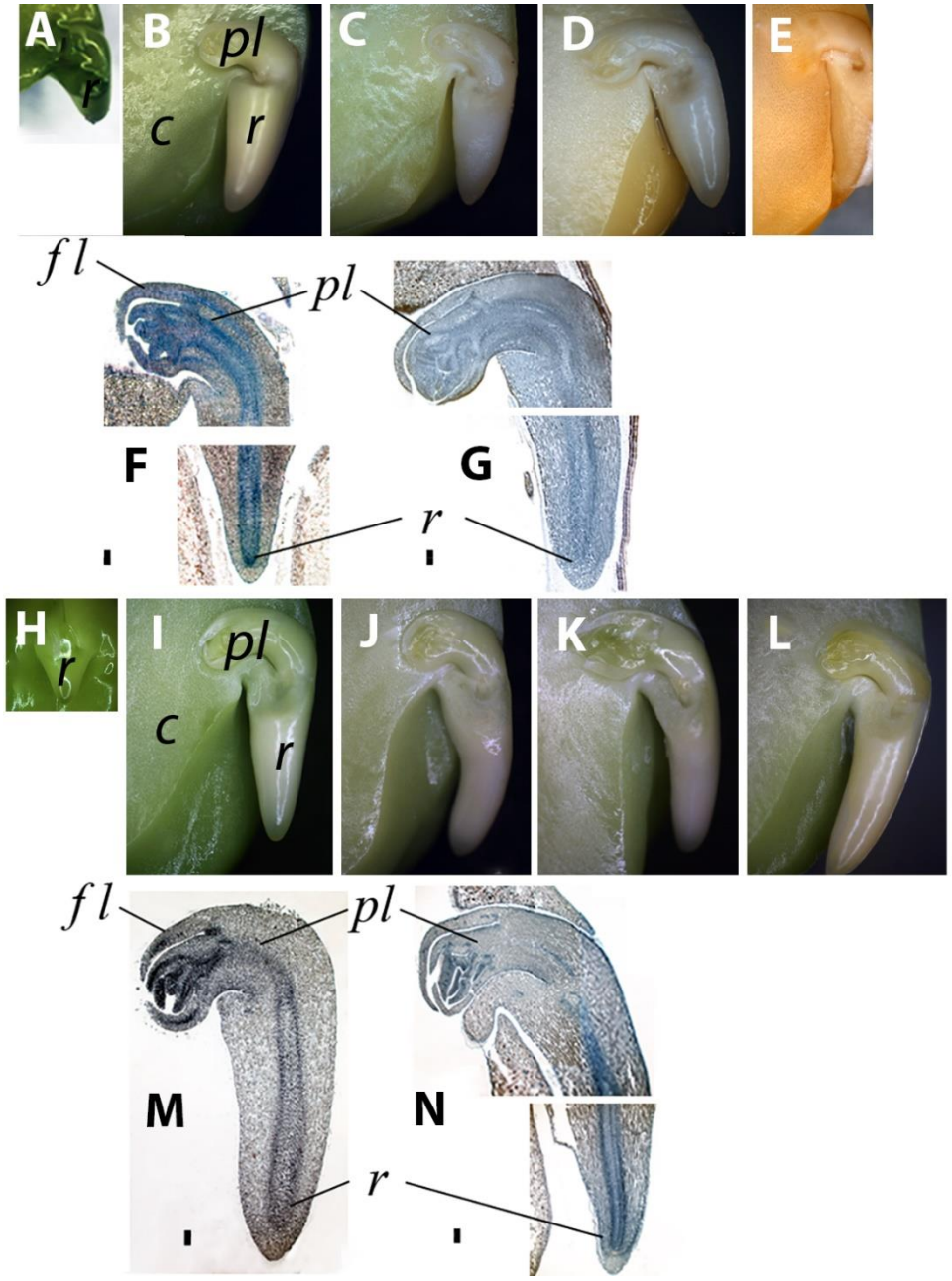

Figure S3 Longitudinal sections of axes of developing pea seeds probed by light microscopy (**A-E, H-L)** and transmission electron microscopy (**F, G, M, N**).

Frisson (yellow-seeded cultivar):

A, F– 16 DAP, **B** – 18 DAP, **C, G**– 24 DAP, **D** – 35 DAP, **E** –mature seed.

Rondo (green-seeded cultivar):

H, M– 16 DAP, **I** – 21 DAP, **J, N** – 24 DAP, **K** – 35 DAP, **L** –mature seed.

DAP – days after pollination, ***c*** – cotyledons, ***f1*** – first leaf, ***pl*** – plumule, ***r*** – radicle.

Scale: **A-E, H-N** – 0.4 mm, **F, G, M, N**– 50 µm.

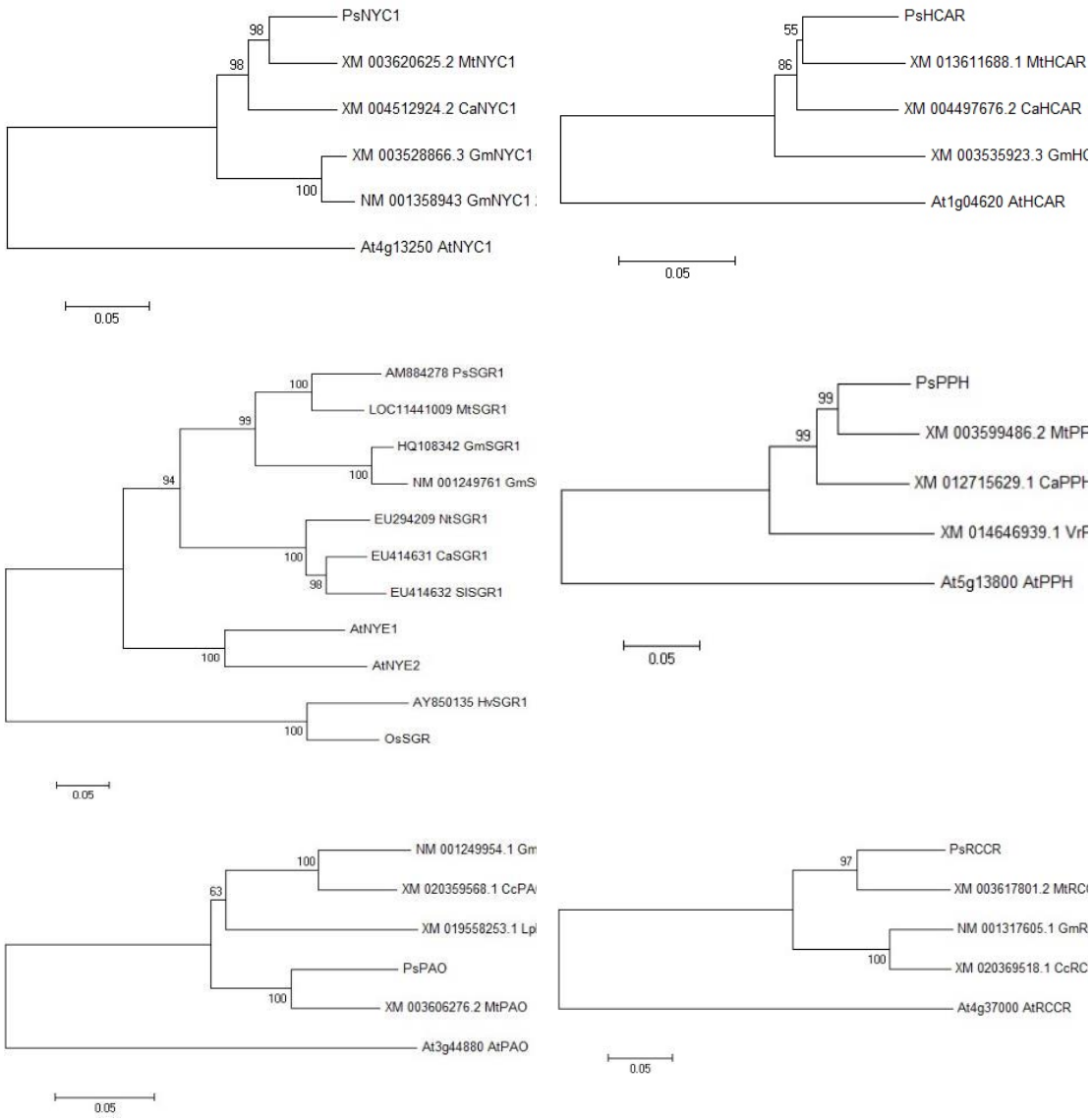

Figure S4 Phylogenetic trees of chlorophyll degradation genes (represented in Figure 8). ***NYC1*** (*NON-YELLOW COLORING 1)* encodes chlorophyll *b* reductase, converting chlorophyll *b* to 7-hydroxymethyl chlorophyll *a*; ***HCAR*** encodes 7-hydroxymethyl chlorophyll *a* reductase, converting 7-hydroxymethyl chlorophyll *a* to chlorophyll *a*; ***SGR*** genes (*STAY-GREEN*) encodes magnesium (Mg)-dechelatase, converting chlorophyll *a* to pheophytin *a; **PPH*** encodes pheophytin pheophorbide hydrolase/pheophytinase, converting pheophytin *a* to pheophorbide *a; **PAO*** encodes pheophorbide *a* oxygenase, which catalyzes oxidative ring opening in pheophorbide *a* and convert it to red chlorophyll catabolite (RCC); ***RCCR*** encodes red chlorophyll catabolite reductase that reduces RCC to FCC (fluorescent red chlorophyll catabolite). The Molecular Evolutionary Genetics Analysis Version 6.0 (MEGA6) software was used to generate graphic output of phylogenetic trees.