

Supporting information

Different Inhibitory Effects of Erythromycin and Chlortetracycline on Early Growth of *Brassica campestris* Seedlings

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Supplementary Materials

Figure S1. The effect of veterinary antibiotics, Ery and CTC, on growth of *Brassica campestris* seedlings in the dark.

Figure S2. Comparison of the effects of different concentrations of Ery on *Brassica campestris* seedlings grown in long-day photoperiods and darkness.

Figure S3. The chlorophyll biosynthesis pathway and the chlorophyll cycle.

Figure S4. Effect of veterinary antibiotics on expression of genes involved in the regulation of chlorophyll content.

Figure S5. Effect of veterinary antibiotics on expression of genes such as *SCO1*, and *RPL21*.

Table S1. List of primer sequences used in this study.

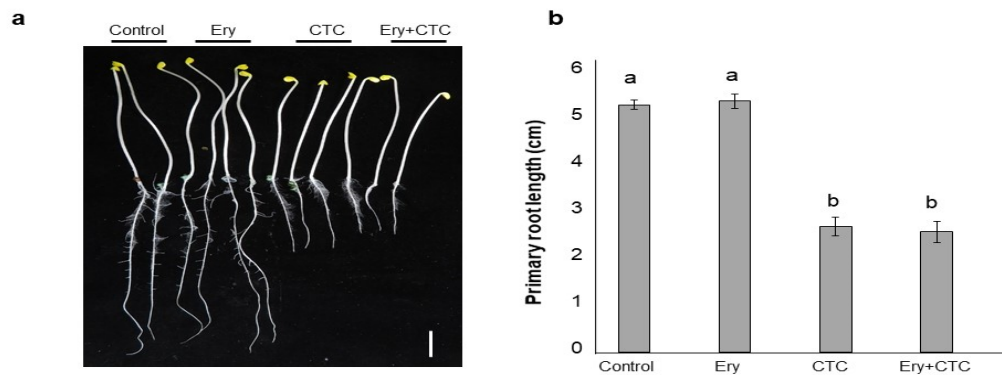


Figure S1. The effect of veterinary antibiotics, Ery and CTC, on growth of *Brassica campestris* seedlings in the dark. Sterilized seeds were placed on Ery, CTC, or Ery/CTC-containing agar medium and grown vertically under continuous dark for five days. (a) Morphological phenotype at day 5. White bar=1 cm (b) Primary root length. Data represent mean \pm SD ($n = 30$). All experiments were replicated three times with similar results by using 5 mg/L indicated veterinary antibiotics. Different letters above bars indicate statistically significant difference within each treatment as determined by one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD), $p < 0.05$.

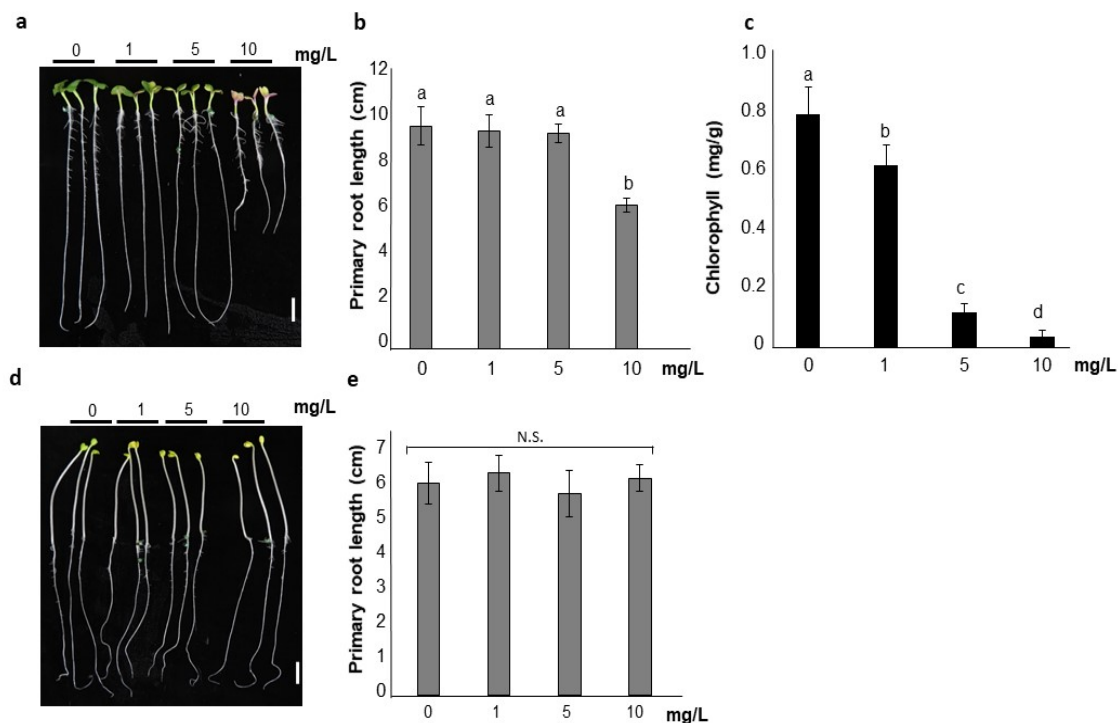


Figure S2. Comparison of the effects of different concentrations of Ery on *B. campestris* seedlings grown in long-day photoperiods and darkness. Sterilized seeds were placed on indicated Ery concentration (0, 1, 5, and 10 mg/L) -containing medium and grown vertically for four days. (a) Morphological phenotype at day 4 grown under long day condition (16 h light/8 h dark). (b) Primary root length of a. Data represent mean \pm SD ($n = 42$). (c) Chlorophyll contents extracted from cotyledon leaves of a. Data represent mean \pm SD ($n=18$). (d) Morphological phenotype at day 4 grown under continuous dark. (e) Primary root length of d. Data represent mean \pm SD ($n = 45$). All experiments were replicated three times with similar results. Different letters above bars indicate statistically significant difference within each treatment as determined by one-way analysis of variance (ANOVA) and Turkey's honestly significant difference (HSD), $p < 0.05$. N.D.=no significantly differences among treatments.

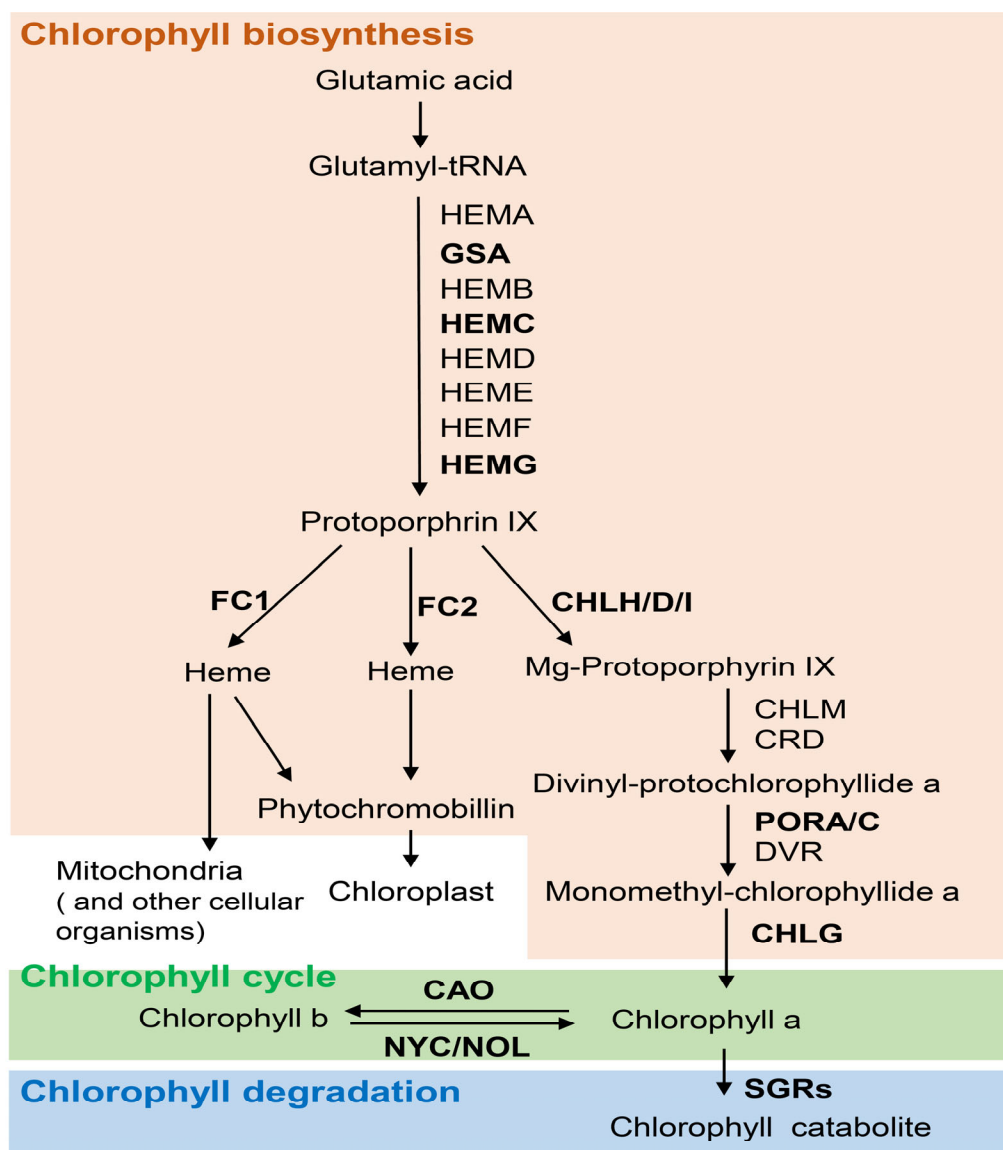


Figure S3. The chlorophyll biosynthesis pathway and the chlorophyll cycle. HEMA: glutamyl-tRNA reductase; GSA: glutamate-1-semialdehyde 2,1-aminomutase; HEMB: glutamate-1-semialdehyde 2,1-aminomutase; HEMC: glutamate-1-semialdehyde 2,1-aminomutase; HEMD: uroporphyrinogen-III synthase; HEME: uroporphyrinogen decarboxylase; HEMF: coproporphyrinogen III oxidase; HEMG: oxygen-dependent protoporphyrinogen oxidase; CHLH: magnesium chelatase subunit H; CHLD: magnesium chelatase subunit D; CHLI: magnesium chelatase subunit I; FC1: ferrochelatase 1; FC2: ferrochelatase 2; CHLM: magnesium-protoporphyrin IX methyltransferase; CRD: magnesium-protoporphyrin IX monomethyl ester cyclase; PORA: protochlorophyllide reductase A; PORC: protochlorophyllide reductase C; DVR: divinyl chlorophyllide a 8-vinyl-reductase; CHLG: chlorophyll synthase; CAO: chlorophyllide a oxygenase; NYC: non-yellow coloring; NOL: non-yellow coloring-like; SGR: stay-green. Expression levels of the genes marked in bold font are shown in Figure S4 and Figure 3.

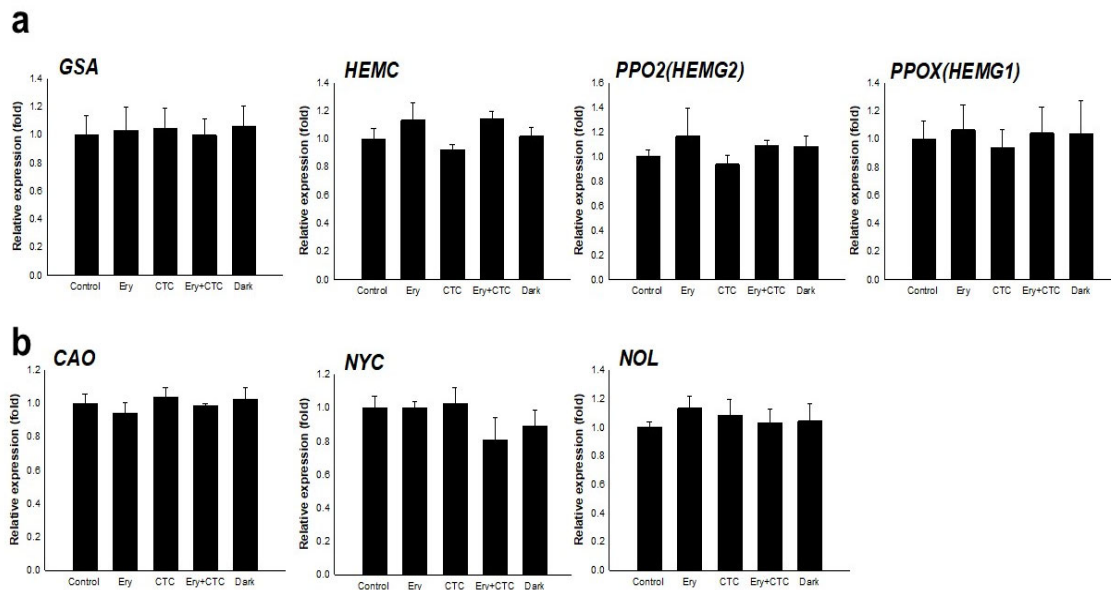


Figure S4. Effect of veterinary antibiotics on expression of genes involved in the protoporphyrin IX biosynthesis and chlorophyll conversion. regulation of chlorophyll content. Expression levels of the genes marked in bold in Figure 3 were determined by qRT-PCR analysis of total RNA extracted from *B. campestris* seedlings grown in the light for 5 days under the following conditions: Control: No antibiotics; Ery: 5 mg/L erythromycin; CTC: 5 mg/L chlortetracycline; Ery+CTC: 5 mg/L erythromycin and 5 mg/L chlortetracycline; Dark: No antibiotics and complete darkness for 5 days. (a) Expression of the protoporphyrin IX biosynthetic genes; **GSA** (*Bra035836* and *Bra038646*), **HEMC** (*Bra007749*), **PPOX** (*HEMG1* and *Bra033317*), and **PPO** (*HEMG2* and *Bra008772*). (b) Expression of the chlorophyll convergent genes; **CAO** (*Bra036948*), **NYC** (*Bra034882* and *Bra032697*), and **NOL** (*Bra005827*). Expression of each gene was normalized against expression of the reference gene *EF1a*. Bars represent means \pm SD ($n = 20$); the experiments were replicated three times with similar results. Different letters above bars indicate statistically significant difference within each treatment as determined by one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD), $p < 0.05$. All data are not significantly differences among treatments.

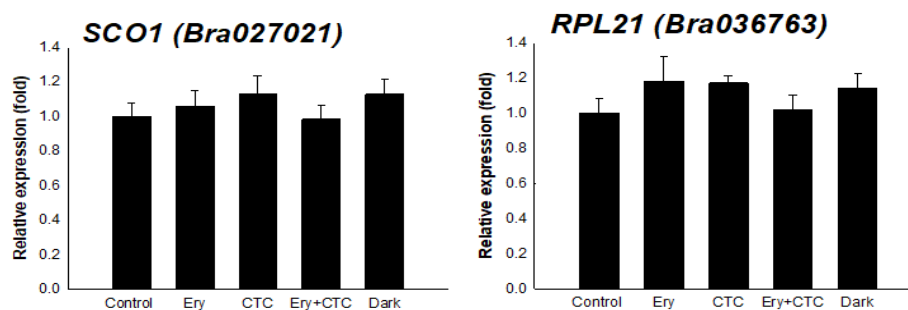


Figure S5. Effect of veterinary antibiotics on expression of *SCO1*, *RPL21*. *SCO1* (*Bra027021*, snowy cotyledons 1) and *RPL21* (*Bra036732*, Ribosomal protein L21) play roles in protein synthesis. Total RNAs were extracted from *B. campestris* seedlings treating indicated conditions. The relative transcript level of *SCO1* and *RPL21* was analyzed by quantitative real-time PCR (qRT-PCR). The expression of genes was normalized to that of *EF1a*. The values are means \pm SD ($n=12$) and three-time replicates are showed similar results. Different letters above bars indicate statistically significant difference within each treatment as determined by one-way analysis of variance (ANOVA) and Turkey's honestly significant difference (HSD), $p < 0.05$. All data are not significantly differences among treatments.

Table S1. List of primer sequences used in this study.

Gene	Accession	Sequence (5'→3')		
		Forward	Reverse	
<i>EF1a</i>		CCCTCCGTCTACCACTTCAG	CACAACCATACCAGGCTTGA	Normalization
<i>GSA</i>	<i>Bra035836</i> <i>Bra038646</i>	GAAGGTGTTTACTTTGCACC	GTGAGCCAAGCTAGTAAACC	Tetrapyrrole biosynthetic pathway
<i>HEMC</i>	<i>Bra007749</i>	GTTGCCTGCATTGGAGAGAC	TCCATGATGCTGTCGACCCA	
<i>HEMG1</i>	<i>Bra033317</i>	GCCTCAAGCCATTCTCTCAGT	CCAGACGACGAGAGAGATGC	
<i>HEMG2</i>	<i>Bra008772</i>	GGGATTGAAGGTGAACCCGT	CCCGCCTCGATGATTACCTG	
<i>FC1</i>	<i>Bra009876</i>	AGCTAGCTTTGGAGTCAGGG	GAACCGAGCCGAAGAACATC	Fe-Chelataase
<i>FC2</i>	<i>Bra007748</i>	TGGTATCAGCGGGAGGGATA	CAGGTCCAACCTCTGCTCTGA	
<i>IM</i>	<i>Bra013604</i>	AAGAAATCGCCTCCACCTGA	GCTCTCATCGTCTTGCAGTG	
<i>CHLH</i>	<i>Bra006208</i>	CTGGTGGTGATGGACAACGA	CCGGTCTAGCCCAATCATC	Mg-Chelataase
<i>CHLD</i>	<i>Bra018619</i>	TGGATGCAACCCCTTAGAGCG	CCAGCTTTCCCTTGCCATTCTG	
<i>CHLI</i>	<i>Bra012595</i> <i>Bra013314</i>	TGTTGATGGGTTGAGAGGAG	CATCTGCAGTCACTCTATCTT	
<i>PORA</i>	<i>Bra003004</i>	GGAACAAGACCTCGGCTTCA	CCACGAGCTTCTCGCTAACT	Chlorophyll biosynthesis
<i>PORC</i>	<i>Bra033415</i> <i>Bra030540</i>	TACCCTGGTTGCATCGCTACA	TCACTCACAACCTGTGCTAG	
<i>CHLG1</i>	<i>Bra040893</i>	GCTTTGGGAGGGTCCTTGTT	ATCGCTATTCCCAACCCAGC	
<i>CHLG2</i>	<i>Bra028411</i>	GTCCAAACCCGATTTTCGCTG	GTAAGTGACCCGGAAGGGAC	
<i>CAO</i>	<i>Bra036948</i>	GTCCTCTTGATCTCGGCTCG	AGGTCCATTACAAGCTCGGC	Chlorophyll cycle
<i>NYC1</i>	<i>Bra034882</i> <i>Bra032697</i>	GATGACCAAGGACGGGCGTT	GTATTCTCTGTATACATCTCC	
<i>NOL</i>	<i>Bra005827</i>	CTCTCCCCTTGTAACGGTCTG	GTCATCGGCTCTCTTGTTCC	
<i>SGR1</i>	<i>Bra019346</i>	TCAGTTGCAAGGATGGGCAA	CCAAAGGGGACCCCAACATT	Chlorophyll degradation
<i>SGR2</i>	<i>Bra000755</i>			
<i>RBCL</i>	<i>Bra028087</i>	TTGCCGAGATAATGGCCTAC	TCATCGCGCAGTAAATCAAC	Photosynthesis
<i>LHCB3.1</i>	<i>Bra002999</i>	TGAAGGCGGTTTGGACTACT	GCGAAAGTAACCGGGTCATC	
<i>LHCB4.2</i>	<i>Bra029732</i>	GGTTTCCGACATTAGCTCCA	TGCCTAAACCAAAAGGATCG	
<i>PSBP-1</i>	<i>Bra031534</i>	GATGGGTTCAAAGTGCAGGT	TTCGGGAGAACCCTAATCAG	
<i>PETC</i>	<i>Bra000837</i>	CTACCTTCTTTGCCCCTCCT	ATGGCACAACACATCCAAGA	
<i>PETC</i>	<i>Bra034200</i>	ATGCCCTTGGAACGATGTA	TGGCACAACACATCCAAGAT	
<i>PSAD-1</i>	<i>Bra036240</i>	GAAGGTCCGAACCTTGCTGAA	CTCCGGATAAACCCCATCTT	
<i>SCO1</i>	<i>Bra027021</i>	TGGAAGCGGGATCAGGATAC	AACAAGCACGGACATCAACC	Protein synthesis
<i>PL21</i>	<i>Bra036763</i>	ATCTCTACACTCAGCGGCTC	CTCGTATTGGCTGTCGGTG	
<i>RBOHD</i>	<i>Bra037520</i> <i>Bra020724</i>	TGGGTGACTAGGGAACAAGG	CATCAACACCGTTCTTAGCG	Oxidative stress
<i>RBOHF</i>	<i>Bra027764</i>	GAACAGCACAGGAAGCAACA	GCATCTCTATCACACCCCGT	
<i>FSD1</i>	<i>Bra013863</i>	ACTCCCAATGCTGTGAATCC	GGCACTTACAGCTTCCCAAG	

<i>FSD2</i>	<i>Bra029190</i>	CCAACGGAGGAAGACAAGAA	GTTTCCCATGACACGAGCTT
	<i>Bra035724</i>		
<i>FSD3</i>	<i>Bra026503</i>	GGATGTGTGGGAGCACTCTT	GATTGGGATGTTGGGTTTAC
	<i>Bra009670</i>		
<i>CSD3</i>	<i>Bra002133</i>	ACGGTGTGCTGAAATCT	CCGCAGATGATTGAAGTCCG
