

On-line Suppl. Tab. 1. Sequences of primer sets used for RT-qPCR to validate results of transcriptome analysis.

Contig name		Primer sequence
Py2K_unigene_122397	F	5'-GAAGATTACCAAGAAGAGCGGC-3'
	R	5'-ACTTTGTCTCGTCCTTCCACTC-3'
Py2K_unigene_173936	F	5'-GCTACAAGATCAAGCCATTCGT-3'
	R	5'-TCAGAACCACCAACAACTCAC-3'
Py2K_unigene_43753	F	5'-CAAGACGGACCATAATCGATACG-3'
	R	5'-AAGGCACCCAAGTACGATGAAT-3'
PyWT_unigene_402	F	5'-AACAGCCATCAATTACCGTCAC-3'
	R	5'-ACAGTCTTCCAGGTTTCAGTCT-3'
Py2K_unigene_145511	F	5'-GCCATGGTCGAGTTCCTCTA-3'
	R	5'-GATGACACCACCCGAGTAGAA-3'
Py2K_unigene_90598	F	5'-GCCATAGGATTAGTAGTAGTTGCA-3'
	R	5'-CTTGCAACTCACCACTTTTCG-3'
Py2K_unigene_146357	F	5'-TCTGAAGCCTATGTTGAGAGCA-3'
	R	5'-TCTTGAACTCATACCACTCCGT-3'
Py2K_unigene_159094	F	5'-ATTTTATGCCGTGGTTAGTGGG-3'
	R	5'-AGGGAATTTATCAAACACGCCA-3'
Py2K_unigene_115858	F	5'-CCATTCCGTCCTGTACAACAC-3'
	R	5'-CCCATTCTTCTGTAGGTCTTGC-3'
Py2K_unigene_159070	F	5'-GGTGTGGACGAGCGACAATACAG-3'
	R	5'-CGGACGGGGGTAAGCAAAGAAG-3'

On-line Suppl. Tab. 2. Summary statistics of raw and clean reads in *Pyropia yezoensis* wild type (PyWT) and mutant (Py2K).

Sample	Raw reads				Clean reads			
	Reads (no.)	Bases (total)	Avg. length (bp)	GC (%)	Reads (no.)	% of raw data	GC (%)	
PyWT	WT1	75,146,820	11,347,169,820	151	45.2	49,337,074	65.65	45.08
	WT2	69,260,700	10,458,365,700	151	44.41	50,952,456	73.57	44.03
	WT3	64,414,678	9,726,616,378	151	44.41	45,643,162	70.86	43.27
	2K1	65,639,484	9,911,562,084	151	44.59	54,125,262	82.46	44.31
Py2K	2K2	70,666,770	10,670,682,270	151	43.16	56,377,874	79.78	42.85
	2K3	79,066,906	11,939,102,806	151	44.02	53,554,388	67.73	44.56

On-line Suppl. Tab. 3. List of upregulated genes in mutant *Pyropia yezoensis* (Py2K) annotated as 'antioxidant'.

TRINITY name	Log ₂ -Fold	Description	Identity	Pfam description
TRINITY_Py2K_DN131974_c0_g1	7.97	Chloroplastic putative glutathione peroxidase 7 [<i>Arabidopsis thaliana</i>]	52.17%	Glutathione peroxidase
TRINITY_Py2K_DN254273_c0_g1	4.28	Glutathione peroxidase-like peroxiredoxin gpx1 [<i>Schizosaccharomyces pombe</i>]	57.14%	Glutathione peroxidase
TRINITY_Py2K_DN63293_c0_g1	6.42	Glutathione S-transferase [<i>Aplysia californica</i>]	32.94%	Glutathione S-transferase, N-terminal domain
TRINITY_Py2K_DN59285_c0_g4	2.53	1-Cys peroxiredoxin [<i>Dictyostelium discoideum</i>]	49.28%	C-terminal domain of 1-Cys peroxiredoxin
TRINITY_Py2K_DN64707_c0_g1	7.55	Glutathione synthetase [<i>Bordetella pertussis</i>]	66.12%	Glutathione synthetase, ATP-grasp domain
TRINITY_Py2K_DN59959_c0_g1	3.74	Chloroplastic 2-Cys peroxiredoxin BAS1 [<i>Hordeum vulgare</i>]	38.39%	C-terminal domain of 1-Cys peroxiredoxin
TRINITY_Py2K_DN51886_c0_g1	4.60	Catalase [<i>Pyropia yezoensis</i>]	91.20%	Catalase
TRINITY_Py2K_DN66210_c7_g3	2.71	Thioredoxin [<i>Porphyra purpurea</i>]	40.00%	Thioredoxin
TRINITY_Py2K_DN65307_c1_g2	4.79	Thioredoxin [<i>Ictalurus punctatus</i>]	46.79%	Thioredoxin
TRINITY_Py2K_DN59105_c0_g1	4.97	Mitochondrial aldehyde dehydrogenase [<i>Pongo abelii</i>]	55.51%	Aldehyde dehydrogenase family

On-line Suppl. Tab. 4. List of differentially expressed genes in *Pyropia yezoensis* mutant (Py2K) related to the Shikimate pathway.

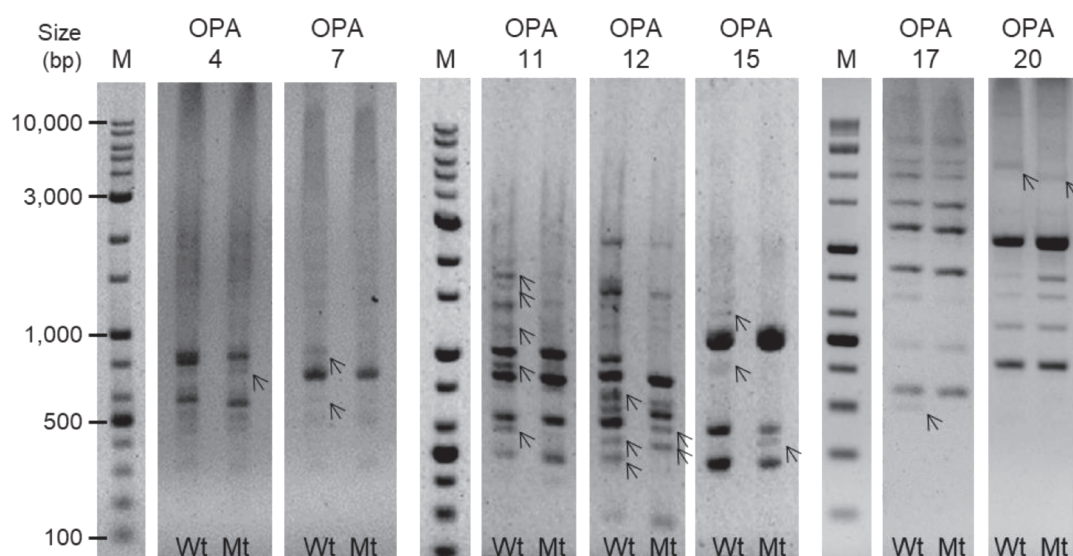
TRINITY name	Log ₂ -Fold	Description	Identity	Pfam description
TRINITY_Py2K_DN61445_c1_g1	2.76	Chorismate synthase [<i>Ruegeria pomeroyi</i>]	83.33%	Chorismate_synt
TRINITY_Py2K_DN61820_c0_g2	4.87	3-dehydroquininate synthase [<i>Thioalkalivibrio sulfidiphilus</i>]	65.69%	3-dehydroquininate synthase
TRINITY_Py2K_DN61821_c0_g1	4.02	Shikimate kinase [<i>Pseudomonas aeruginosa</i>]	60.00%	Shikimate kinase
TRINITY_Py2K_DN63681_c0_g1	6.94	Shikimate dehydrogenase [<i>Methylobacillus flagellatus</i>]	60.44%	Shikimate dehydrogenase substrate binding domain

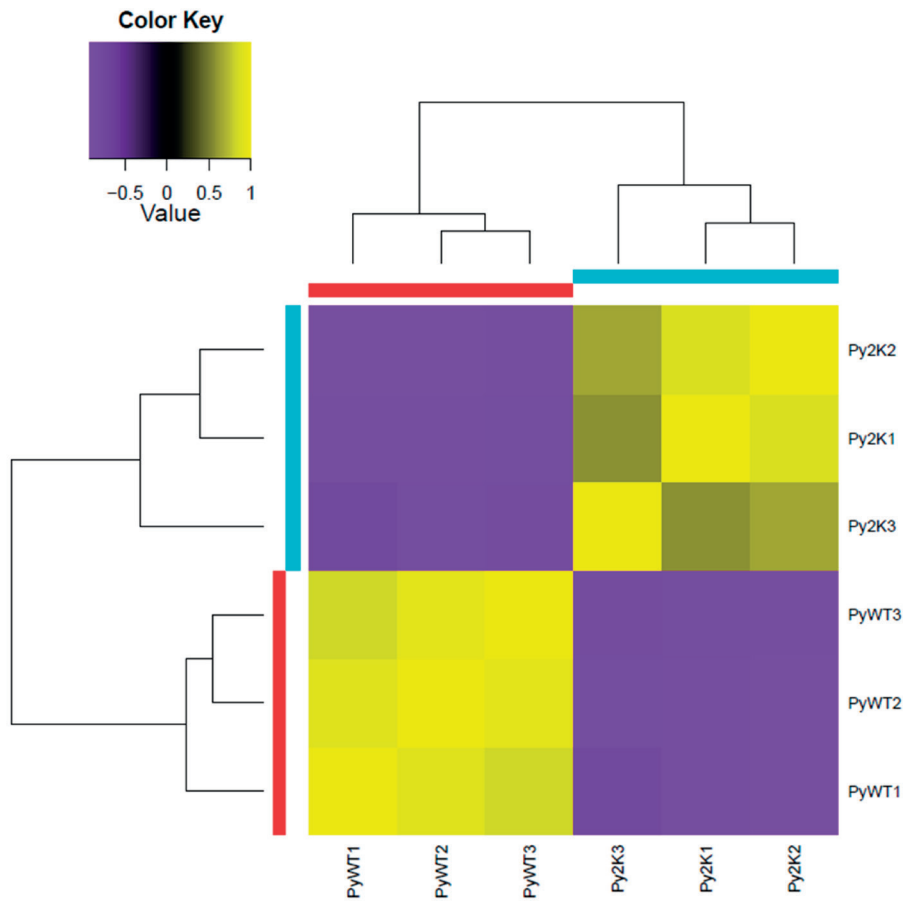
On-line Suppl. Tab. 5. List of differentially expressed genes in *Pyropia yezoensis* mutant (Py2K) related to nitrogen assimilation and re-assimilation, ribosome biogenesis, and amino acid biosynthesis.

	TRINITY name	log ₂ Fold	Description	Identity	Pfam description
Nitrogen assimilation	TRINITY_Py2K_DN59400_c0_g2	4.22	High affinity nitrate transporter 2.5 [<i>Pyropia yezoensis</i>]	99.20%	Major Facilitator Super-family
	TRINITY_PyWT_DN101823_c0_g1	2.76	Chloroplastic ferredoxin-nitrite reductase [<i>Arabidopsis thaliana</i>]	28.94%	Nitrite and sulfite reductase 4Fe-4S domain
	TRINITY_Py2K_DN61283_c1_g2	2.87	Glutamine synthetase [<i>Cryptococcus neoformans</i> var. <i>neoformans</i> serotype D]	56.34%	Glutamine synthetase, beta-Grasp domain
Ribosome biogenesis	TRINITY_Py2K_DN141935_c0_g1	7.05	Casein kinase II subunit beta [<i>Arabidopsis thaliana</i>]	61.28%	Casein kinase II regulatory subunit
	TRINITY_Py2K_DN278700_c0_g1	6.53	Serine/threonine-protein kinase rio1 [<i>Capsaspora owczarzewski</i> ATCC 30864]	92.40%	Calcineurin-like phospho-esterase
	TRINITY_Py2K_DN56816_c1_g1	6.97	GTP-binding nuclear protein Ran [<i>Sphaeroforma arctica</i> JP610]	74.50%	ADP-ribosylation factor family
	TRINITY_Py2K_DN303240_c0_g1	6.68	13 kDa ribonucleoprotein-associated protein [<i>Nicotiana tomentosiformis</i>]	76.00%	Ribosomal protein L7Ae/L30e/S12e/Gadd45 family
	TRINITY_Py2K_DN63805_c1_g4	5.18	Oligoribonuclease [<i>Pseudomonas aeruginosa</i>]	67.98%	Exonuclease
	TRINITY_Py2K_DN19438_c0_g1	6.26	NTF2-related export protein [<i>Caenorhabditis elegans</i>]	28.57%	Nuclear transport factor 2 (NTF2) domain
	TRINITY_Py2K_DN56108_c0_g1	6.42	Guanine nucleotide-binding protein-like 3 homolog [<i>Dictyostelium discoideum</i>]	45.45%	GNL3L/Grn1 putative GTPase
	TRINITY_Py2K_DN292600_c0_g1	7.81	Nucleolar protein 56 [<i>Schizosaccharomyces pombe</i>]	50.62%	NOP5NT (NUC127) domain
Purine ring catabolism	TRINITY_Py2K_DN50780_c0_g1	6.37	Probable allantoinase 1 [<i>Dictyostelium discoideum</i>]	40.86%	OHCU decarboxylase
	TRINITY_Py2K_DN63532_c0_g1	4.69	Probable xanthine dehydrogenase subunit A [<i>Bacillus subtilis</i>]	27.03%	XdhC and CoxI family
Biosynthesis of amino acids	TRINITY_Py2K_DN203287_c0_g1	2.61	D-amino-acid N-acetyltransferase [<i>Saccharomyces cerevisiae</i>]	37.93%	Acetyltransferase (GNAT) family
	TRINITY_Py2K_DN251932_c0_g1	3.69	Cystathionine gamma-lyase [<i>Dictyostelium discoideum</i>]	57.89%	Cys/Met metabolism PLP-dependent enzyme
	TRINITY_Py2K_DN33143_c0_g2	6.30	Argininosuccinate synthase [<i>Achlya hypogyna</i>]	58.80%	Argininosuccinate synthase

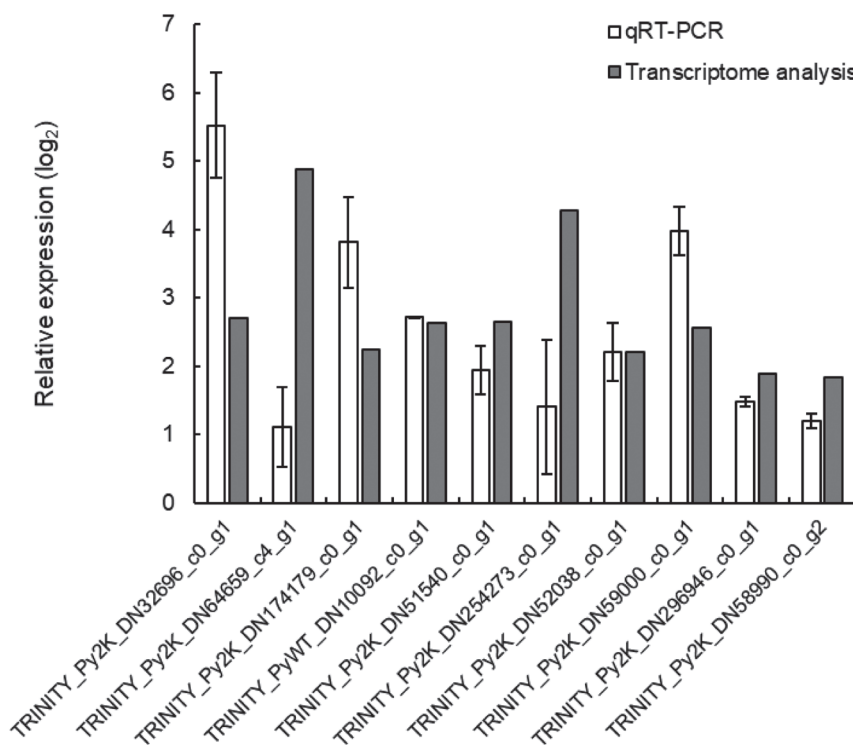
On-line Suppl. Tab. 6. List of differentially expressed genes in *Pyropia yezoensis* mutant (Py2K) related to the electron transport chain.

TRINITY name	log ₂ Fold	Description	Identity	Pfam description
TRINITY_Py2K_DN188135_c0_g1	2.41	NADH dehydrogenase ubiquinone iron-sulfur protein 8-B [<i>Arabidopsis thaliana</i>]	61.19%	4Fe-4S binding domain
TRINITY_Py2K_DN63881_c0_g1	3.02	Succinate dehydrogenase assembly factor 2 [<i>Dictyostelium discoideum</i>]	39.77%	Flavinator of succinate dehydrogenase
TRINITY_Py2K_DN63855_c0_g2	2.50	Mitochondrial succinate dehydrogenase ubiquinone flavoprotein subunit [<i>Oryza sativa</i> subsp. Japonica]	74.75%	Fumarate reductase flavoprotein C-term
TRINITY_Py2K_DN65270_c1_g1	5.93	Alternative oxidase [<i>Mangifera indica</i>]	59.60%	Alternative oxidase
TRINITY_Py2K_DN38046_c0_g1	6.50	Cytochrome b-c1 complex subunit Rieske-1 [<i>Arabidopsis thaliana</i>]	65.22%	Ubiquinol cytochrome reductase transmembrane region
TRINITY_Py2K_DN65488_c2_g5	8.24	Ubiquinol-cytochrome c reductase iron-sulfur subunit [<i>Allochromatium vinosum</i>]	56.68%	Ubiquinol-cytochrome C reductase Fe-S subunit TAT signal
TRINITY_Py2K_DN28456_c0_g1	2.46	Cytochrome c [<i>Pectinaria gouldii</i>]	71.96%	Cytochrome c
TRINITY_Py2K_DN63840_c0_g2	2.11	Cytochrome c oxidase subunit 1 [<i>Marchantia polymorpha</i>]	76.81%	Cytochrome C and Quinol oxidase polypeptide
TRINITY_Py2K_DN3622_c0_g1	2.29	Mitochondrial ATP synthase subunit beta [<i>Fistulifera solaris</i>]	84.10%	ATP synthase alpha/beta family, beta-barrel domain
TRINITY_Py2K_DN61343_c0_g1	5.44	Mitochondrial isocitrate dehydrogenase [<i>Candida tropicalis</i>]	67.74%	Isocitrate/isopropylmalate dehydrogenase
TRINITY_Py2K_DN129036_c0_g1	5.79	Putative cytochrome b5 [<i>Neurospora crassa</i>]	45.45%	Cytochrome b5-like Heme/Steroid binding domain
TRINITY_Py2K_DN62822_c0_g1	2.55	Mitochondrial manganese-dependent superoxide dismutase [<i>Dictyostelium discoideum</i>]	58.26%	Iron/manganese superoxide dismutases, alpha-hairpin domain

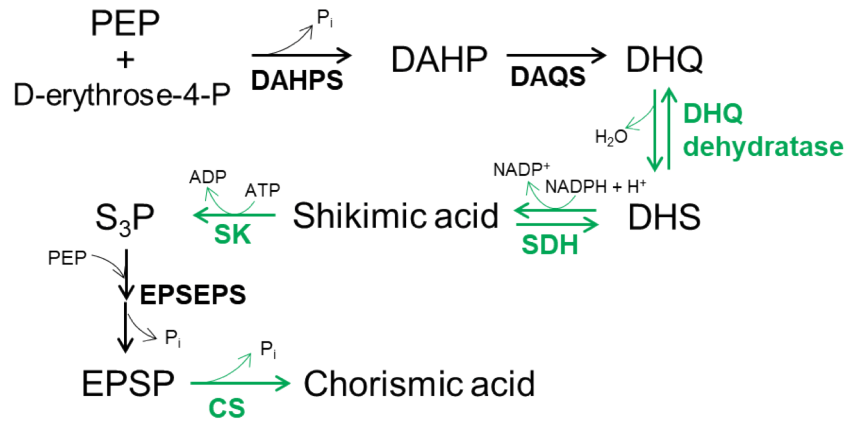
**On-line Suppl. Fig. 1.** Random amplified polymorphic DNA (RAPD) analysis of *Pyropia yezoensis* wild-type (PyWT) and mutant (Py2K). RAPD analysis was conducted to compare PyWT and Py2K using 20 RAPD primer sets (OPA 1-20 primer sets). Representative amplifications with OPA 4, 7, 11, 12, 15, 17 and 20 are shown. Arrows indicate the bands that were different between PyWT and Py2K. Wt, wild-type; Mt, mutant.



On-line Suppl. Fig. 2. Relationship among samples based on log₂-transformed count data between *Pyropia yezoensis* wild-type (PyWT) and mutant (Py2K) with hierarchically clustered heatmap using Pearson's correlation coefficients.



On-line Suppl. Fig. 3. Reverse transcription quantitative PCR (RT-qPCR) results for verification of transcriptome analysis results. All experiments were performed in triplicate and error bar indicated standard deviation.



On-line Suppl. Fig. 4. Description of differentially expressed genes (DEGs) in the shikimate biosynthesis pathway related to phenol biosynthesis in Py2K. The shikimate pathway was described by Santos-Sánchez et al. (2019). The green arrows indicate upregulated genes in Py2K. PEP – phosphoenolpyruvic acid, D-erythrose-4-P – D-erythrose-4-phosphate, DAHP – 3-deoxy-D-arabion-heptulosonic acid 7-phosphate, DHQ – 3-dehydroquinic acid, DHS – 3-dehydroshikimic acid, S₃P – shikimic acid 3-phosphate, EPSP – 5-enolpyruvylshikimic acid 3-phosphate, DAHPS – 3-deoxy-D-arabion-heptulosonic acid 7-phosphate synthase, DHQS – 3-dehydroquinic acid synthase, SDH – shikimate dehydrogenase, SK – shikimate kinase, EPSPS – 5-enolpyruvylshikimic acid 3-phosphate synthase, CS – chorismite synthase.