

Supplementary Material

Specific physiological responses to alkaline carbonate stress in rice (*Oryza sativa*) seedlings: organic acid metabolism and hormone signalling

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Table S1. Primer sequences used for qPCR validation

Gene ID	Forward primer (5'to 3')	Reverse primer (5'to 3')
gene-LOC4325145	ATGGGAGGTTCAAGAGCGTG	TGGCAATGGTGCAATCCTCT
gene-LOC4341343	CAGCGACTACGGTGACTACC	TCCTCTCTCTTGTAACCGCGA
gene-LOC4331834	TTCCAAGTTCCAACCCTCCAC	TCTTGTTCTTCCTCGCTGGT
gene-LOC4332374	CTCGTCAGACAGATCAGCCC	CGAGCAAAGGGGACACTCAA
gene-LOC4348533	GGCCCAAACCCAAAGAGTTC	GCCAGTACACATGACAACAGT
gene-LOC4331833	CAGGTGAACCCCACTCTCAG	CCCCCGGAAGAAACCAACAA
gene-LOC4333065	TTGTTAGTCTCGTGCGTCCC	CGAAATCCTCCCCTTCGAT
gene-LOC9268838	TCTGTCTGCAGGCACTATCT	GCAGCTAGCAGCCAAGACAT
gene-LOC4339092	AGGACTCCATGCTAATGGCT	GCCTAGTGTCAGCGTCCAT
gene-LOC4331832	GTTCTCGTCGCATTAACGGC	CGCCGCGATAACTAGGGTAA
gene-LOC4335698	AGCAAAGGACTCGCTCGTG	TACGGACCCAAGCTGGTCAA
gene-LOC4348531	CGCCAAGAAGAAATTAACCCGA	GATCAAAATCAACCAGCCGC
<i>OsActin</i>	TGCTATGTACGTGCCATCCA	AATGAGTAACCACGCTCCGTC

Table S2. Top ten of up-regulated DEGs in rice seedlings under saline-alkaline

stresses compared with control groups

Group	Gene ID	Annotation	log ₂ FC	FPKM	P_value
NaCl vs CK	gene-LOC4346916	RING-H2 finger protein ATL46	5.24	0.73	0.01
	gene-LOC4350827	protein WVD2-like 1	4.81	0.84	0.03
	gene-LOC4345805	uncharacterized protein LOC4345805	4.65	2.88	0.00
	gene-LOC4331375	probable receptor-like protein kinase At1g33260	4.46	3.08	0.00
	gene-LOC4344246	pollen-specific leucine-rich repeat extensin-like protein 2	4.37	2.57	0.00
	gene-LOC4336666	nitrate regulatory gene2 protein	4.27	0.99	0.00
	gene-LOC4349896	BURP domain	4.27	4.92	0.00
	gene-LOC4340634	microtubule-associated protein TORTIFOLIA1	4.22	2.20	0.00
	gene-LOC4332925	peroxidase 2	4.20	3.60	0.00
	gene-LOC4334681	probable glycerol-3-phosphate acyltransferase 3	4.12	5.30	0.00
Na ₂ CO ₃ vs CK	gene-LOC9270547	vegetative cell wall protein gp1	8.24	32.90	0.00
	gene-LOC9268807	tryptophan synthase alpha chain	8.01	134.35	0.00
	gene-LOC9272317	IQ domain-containing protein IQM2	7.95	6.53	0.00
	gene-LOC11293689 9	Os11g0181200	7.86	22.57	0.00
	gene-LOC4325388	G-type lectin S-receptor-like serine/threonine-protein kinase At5g35370	7.85	8.22	0.00
	gene-LOC4348490	transcription factor bHLH96	6.65	4.33	0.01
	gene-LOC4329309	probable WRKY transcription factor 11	6.45	52.11	0.00
	gene-LOC4348531	protein TIFY 11e	6.39	11.99	0.00
	gene-LOC4347345	ABC transporter G family member 11	6.15	1.72	0.03
	gene-LOC4352721	auxin-responsive protein IAA30	5.94	4.47	0.01
NaHCO ₃ vs CK	gene-LOC4349197	probable glutathione S-transferase GSTU6, partial	7.88	13.79	0.00
	gene-LOC4326104	probable receptor-like protein kinase At1g67000 isoform X1	6.76	2.73	0.00
	gene-LOC4348123	proline-rich protein 4	6.44	5.32	0.00
	gene-LOC11293749 4	hypothetical protein OsI_00139	6.30	2.62	0.00
	gene-LOC4349212	transcription factor RF2a-like	6.25	0.97	0.00
	gene-LOC4348595	BURP domain-containing protein	3.62	8.63	0.00

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gene-LOC9272536	auxin-responsive protein SAUR71	3.58	2.45	0.05	
gene-LOC9270209	F-box/kelch-repeat protein At1g23390	3.46	1.66	0.03	
gene-LOC4326102	probable receptor-like protein kinase At5g39020	3.26	2.25	0.00	
gene-LOC4347807	RING-H2 finger protein ATL44	2.02	7.72	0.01	

Note: $\log_2FC = \log_2$ Fold Change.

Table S3. Top ten of up-regulated differentially expressed genes in rice seedlings in different saline-alkaline groups

Groups	Gene ID	Annotation	log ₂ FC	FPKM	P_value
Na ₂ CO ₃ vs NaCl	gene-LOC4338583	probable esterase PIR7A isoform X1	6.96	6.55	0.00
	gene-LOC4334903	probable inactive methyltransferase Os04g0175900	6.58	3.95	0.01
	gene-LOC107275877	probable flavin-containing monooxygenase 1	6.38	2.26	0.02
	gene-LOC107276859	putative EG45-like domain containing protein 1	6.21	4.93	0.02
	gene-LOC107277647	germin-like protein 1-2	5.96	2.92	0.04
	gene-LOC4324859	rop guanine nucleotide exchange factor 7	5.89	1.57	0.05
	gene-LOC4339544	uncharacterized protein	5.89	2.34	0.05
	gene-LOC4339989	uncharacterized protein	5.89	2.17	0.05
	gene-LOC4347195	uncharacterized protein	5.37	3.41	0.03
	gene-LOC4330695	uncharacterized protein	5.14	3.81	0.02
NaHCO ₃ vs NaCl	gene-LOC4332758	valine--tRNA ligase, mitochondrial 1 isoform X1	8.05	3.86	0.00
	gene-LOC4350092	uncharacterized protein	6.11	3.33	0.00
	gene-LOC107279425	uncharacterized protein	5.44	1.10	0.01
	gene-LOC4349212	transcription factor RF2a-like	5.32	0.97	0.00
	gene-LOC4326104	probable receptor-like protein kinase At1g67000 isoform X1	5.24	2.73	0.00
	gene-LOC4334972	subtilisin-like protease SBT3.9	5.23	0.99	0.01
	gene-LOC107276774	wall-associated receptor kinase 5	5.13	0.69	0.02
	gene-LOC4334900	WASH complex subunit CCDC53 homolog	5.11	5.53	0.00
	gene-LOC107278391	GDSL esterase/lipase At4g10955-like	4.30	3.68	0.00
	gene-LOC4338211	xylanase inhibitor protein 2-like	3.69	1.83	0.04
Na ₂ CO ₃ vs NaHCO ₃	gene-LOC4341996	leucine-rich repeat extensin-like protein 2	5.89	0.03	0.01
	gene-LOC4335987	probable fucosyltransferase 7	5.77	0.02	0.02
	gene-LOC4327059	calcium-binding protein PBP1	5.66	0.07	0.03
	gene-LOC9267218	zinc finger protein STOP1 homolog	5.61	0.03	0.03
	gene-LOC107277999	uncharacterized protein	5.52	0.08	0.04
	gene-LOC107277540	putative expansin-A30	4.89	0.07	0.03
	gene-LOC4334789	uncharacterized protein At4g15970	4.72	0.09	0.04
	gene-LOC4335805	non-specific lipid-transfer protein	4.50	0.59	0.00
	gene-LOC4341623	premnaspirodiene oxygenase	4.37	0.18	0.01
gene-LOC4340427	LRR receptor-like serine/threonine-protein kinase ERECTA	3.90	0.07	0.05	

Note: log₂FC = log₂ Fold Change.

Table S4. GO terms significantly enriched among up-regulated DEGs in rice seedlings under salt-alkaline treatments

groups	GO terms	DEGs in the term	Genes in background	significant
NaCl	cell wall organization or biogenesis	25	558	yes
	metal ion homeostasis	8	149	yes
	polysaccharide metabolic process	11	360	yes
	intrinsic component of membrane	70	6684	yes
	cytoskeleton	12	196	yes
	Golgi apparatus	14	480	yes
	transferase activity	18	642	yes
	microtubule motor activity	7	58	yes
	microtubule binding	7	93	yes
Na ₂ CO ₃	jasmonic acid mediated signaling pathway	8	32	yes
	response to acid chemical	19	256	yes
	carboxylic acid metabolic process	44	1050	yes
	intrinsic component of plasma membrane	20	429	yes
	plasma membrane part	21	487	yes
	mitochondrial respiratory chain supercomplex	2	4	yes
	transcription corepressor activity	7	22	yes
	oxidoreductase activity	64	1807	yes
	transcription regulator activity	36	828	yes
	NaHCO ₃	oxidation-reduction process	20	1810
detoxification		7	252	yes
cofactor catabolic process		6	168	yes
extracellular region		10	674	yes
intrinsic component of membrane		45	6684	yes
membrane part		46	6964	yes
antioxidant activity		6	215	yes
peroxidase activity		6	196	yes
oxidoreductase activity		20	1807	yes

Table S5. KEGG enrichment analysis of up-regulated DEGs in rice seedlings under salt-alkaline treatments

Groups	KEGG pathways	DEGs in the pathways	Genes in background	significant
NaCl	Flavone and flavonol biosynthesis	2	4	yes
	Pathogenic Escherichia coli infection	5	127	yes
	Phenylpropanoid biosynthesis	6	251	yes
	Salmonella infection	5	198	yes
	Gap junction	2	16	yes
	Cyanoamino acid metabolism	3	59	yes
	Yersinia infection	3	77	yes
	Bacterial invasion of epithelial cells	2	25	yes
	Starch and sucrose metabolism	4	165	yes
Na ₂ CO ₃	Phenylalanine, tyrosine and tryptophan biosynthesis	9	54	yes
	Plant hormone signal transduction	13	276	yes
	Galactose metabolism	6	72	yes
	Carotenoid biosynthesis	4	33	yes
	Stilbenoid, diarylheptanoid and gingerol biosynthesis	3	20	yes
	Phenylpropanoid biosynthesis	11	251	yes
	Two-component system	4	43	yes
	beta-Alanine metabolism	4	46	yes
	Diterpenoid biosynthesis	4	46	yes
NaHCO ₃	Phenylpropanoid biosynthesis	5	251	yes
	Taste transduction	1	2	yes
	beta-Alanine metabolism	2	46	yes
	Fluid shear stress and atherosclerosis	3	163	yes
	Monoterpenoid biosynthesis	1	10	yes

Table S6. KEGG enriched analysis of organic acid and hormone metabolism in rice seedlings under different stresses.

Treats	ID	Pathway	List	P-value	List_gene	URL
NaCl	map00592	alpha-Linolenic acid metabolism	2	0.0462	gene-LOC4340976(K00232);gene-LOC112936094(K05894)	http://www.kegg.jp/kegg-bin/show_pathway?map00592/K00232%09%23FFFFFFF,red/K05894%09%23FFFFFFF,red
	map00592	alpha-Linolenic acid metabolism	4	0.0326	gene-LOC4338358(K00454);gene-LOC4328040(K10528);gene-LOC4352509(K00454);gene-LOC4328742(K01723)	http://www.kegg.jp/kegg-bin/show_pathway?map00592/K00454%09%23FFFFFFF,red/K10528%09%23FFFFFFF,red/K01723%09%23FFFFFFF,red
Na ₂ CO ₃	map00630	Glyoxylate and dicarboxylate metabolism	7	0.0016	gene-LOC4325932(K00281);gene-LOC4341516(K00281);gene-LOC4331509(K03781);gene-LOC4345962(K00830);gene-LOC4337051(K00605);gene-LOC4349114(K02437);gene-LOC4333896(K01915)	http://www.kegg.jp/kegg-bin/show_pathway?map00630/K00281%09%23FFFFFFF,red/K03781%09%23FFFFFFF,red/K00830%09%23FFFFFFF,red/K00605%09%23FFFFFFF,red/K02437%09%23FFFFFFF,red/K01915%09%23FFFFFFF,red
	map04075	Plant hormone signal transduction	13	0.0089	gene-LOC4352721(K14484);gene-LOC4331834(K13464);gene-LOC4332374(K14497);gene-LOC4348533(K13464);gene-LOC4326893(K14487);gene-LOC4330838(K14432);gene-LOC4343785(K14487);gene-LOC4331833(K13464);gene-LOC4331832(K13464);gene-LOC4335698(K13464);gene-LOC4333065(K13464);gene-LOC9268838(K14497);gene-LOC4348531(K13464)	http://www.kegg.jp/kegg-bin/show_pathway?map04075/K14484%09%23FFFFFFF,red/K13464%09%23FFFFFFF,red/K14497%09%23FFFFFFF,red/K14487%09%23FFFFFFF,red/K14432%09%23FFFFFFF,red
	map00906	Carotenoid biosynthesis	4	0.0060	gene-LOC4349224(K15746);gene-LOC4331460(K15746);gene-LOC4333566(K09840);gene-LOC4342424(K09840)	http://www.kegg.jp/kegg-bin/show_pathway?map00906/K15746%09%23FFFFFFF,red/K09840%09%23FFFFFFF,red
	map00906	Carotenoid biosynthesis	2	0.0376	gene-LOC9270250(K09840);gene-LOC4345810(K09843)	http://www.kegg.jp/kegg-bin/show_pathway?map00906/K09840%09%23FFFFFFF,red/K09843%09%23FFFFFFF,red
NaHCO ₃	map00906	Carotenoid biosynthesis	2	0.0376	gene-LOC9270250(K09840);gene-LOC4345810(K09843)	http://www.kegg.jp/kegg-bin/show_pathway?map00906/K09840%09%23FFFFFFF,red/K09843%09%23FFFFFFF,red

	map04 075	Plant hormone signal transductio n	6	0.0428	gene-LOC4342759(K14516);gene-LOC4330855(K14488);gene-LOC4349747(K14492);gene-LOC4335934(K14486);gene-LOC4329677(K14492);gene-LOC4326541(K14484)	http://www.kegg.jp/kegg-bin/show_pathway?map04075/K14516%09%23FFFFFF,red/K14488%09%23FFFFFF,red/K14492%09%23FFFFFF,red/K14486%09%23FFFFFF,red/K14484%09%23FFFFFF,red
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Table S7. Differentially expressed genes (DEGs) that were upregulated under Na₂CO₃ but no expression under NaCl treatment.

gene ID	Annotation	NaCl			Na CO ₃		
		p_value	up/down	significant	p_value	up/down	significant
gene-LOC107276337	transcription factor UNE12-like isoform X1	-	-	-	4.87E-06	Up	yes
gene-LOC107277108	putative receptor-like protein kinase At3g47110	-	-	-	0.008123	Up	yes
gene-LOC107277295	adenylate isopentenyltransferase 3, chloroplastic-like	-	-	-	0.000792	Up	yes
gene-LOC107277955	LOW QUALITY PROTEIN	-	-	-	0.035548	Up	yes
gene-LOC107278589	uncharacterized protein LOC107278589	-	-	-	0.017303	Up	yes
gene-LOC107278789	uncharacterized protein LOC107278789 isoform X1	-	-	-	0.045735	Up	yes
gene-LOC107280598	AT-rich interactive domain-containing protein 1B-like	-	-	-	0.024056	Up	yes
gene-LOC107281435	uncharacterized protein LOC107281435	-	-	-	0.000555	Up	yes
gene-LOC112937355	hypothetical protein OsI_38292	-	-	-	0.035548	Up	yes
gene-LOC4325945	probable WRKY transcription factor 23	-	-	-	7.49E-06	Up	yes
gene-LOC4327325	uncharacterized protein LOC4327325	-	-	-	0.003877	Up	yes
gene-LOC4329329	cytochrome P450 CYP73A100	-	-	-	0.000111	Up	yes
gene-LOC4329721	cytochrome P450 76M5	-	-	-	0.00605	Up	yes
gene-LOC4330303	hypothetical protein OsI_08452	-	-	-	0.02086	Up	yes
gene-LOC4331350	uncharacterized protein LOC4331350 isoform X1	-	-	-	4.46E-06	Up	yes
gene-LOC4332423	cysteine-rich repeat secretory protein 55	-	-	-	0.032195	Up	yes
gene-LOC4333197	zinc finger protein 1	-	-	-	2.07E-06	Up	yes
gene-LOC4334663	cell number regulator 10	-	-	-	1.50E-08	Up	yes
gene-LOC4335094	syn-pimara-7,15-diene synthase	-	-	-	1.14E-08	Up	yes
gene-LOC4335162	putative disease resistance RPP13-like protein 2	-	-	-	0.029187	Up	yes
gene-LOC4336845	OSJNba0093F12.2	-	-	-	0.000573	Up	yes
gene-LOC4339066	pyruvate decarboxylase 1	-	-	-	2.96E-05	Up	yes
gene-LOC4339500	uncharacterized protein LOC4339500	-	-	-	0.01578	Up	yes
gene-LOC4342734	ent-cassadiene C2-hydroxylase-like	-	-	-	0.000329	Up	yes

gene-LOC4345156	cinnamoyl-CoA reductase 1	-	-	-	0.003584	Up	yes
gene-LOC4346881	polyamine oxidase	-	-	-	0.037367	Up	yes
gene-LOC4347816	butyrate--CoA ligase AAE11, peroxisomal	-	-	-	3.52E-06	Up	yes
gene-LOC4348505	tyrosine decarboxylase 1	-	-	-	0.025238	Up	yes
gene-LOC4348721	ent-cassadiene C2-hydroxylase-like isoform X1	-	-	-	0.029187	Up	yes
gene-LOC4351010	agmatine coumaroyltransferase-1	-	-	-	7.35E-06	Up	yes
gene-LOC9267722	probable flavin-containing monooxygenase 1	-	-	-	0.003315	Up	yes
gene-LOC9268881	Os06g0287700	-	-	-	0.000846	Up	yes
gene-LOC9270657	alpha-humulene synthase isoform X1	-	-	-	0.024056	Up	yes
gene-LOC9270948	probable E3 ubiquitin-protein ligase HERC2	-	-	-	2.02E-07	Up	yes
gene-LOC9271182	agmatine coumaroyltransferase-1	-	-	-	0.025238	Up	yes

Note: Up or down means significantly induced or suppressed.

Table S8. Differentially expressed genes (DEGs) that were upregulated under alkaline stress but down-regulated or no expression under NaCl treatment

gene	Annotation	NaCl					Na ₂ CO ₃					NaHCO ₃				
		log ₂ FC	Fold Change	p-value	up/down	significant	log ₂ FC	Fold Change	p-value	up/down	significant	log ₂ FC	Fold Change	p-value	up/down	significant
gene-L OC432 8018	sucrose 1-fructosyltransferase	-1.95	1.07	0.02	Down	-	3.42	43.46	0.02	Up	yes	1.37	10.11	0.04	Up	yes
		47.3	03.8	29.3			76.5	68.9	86			60.8	12.4	53.5		
gene-L OC435 0807	ABC transporter G family member 48	-3.20	0.150	0.00	Down	-	2.962	11.39	0.011	Up	yes	2.508	7.918	8.21E-08	Up	yes
gene-L OC435 2488	major pollen allergen Bet v 1-D/H	-1.43	6.419	0.05	Down	-	2.715	11.97	0.014	Up	yes	1.026	35.35	0.023	Up	yes
gene-L OC107 277955	LOW QUALITY PROTEIN	-	-	-	-	-	Inf	2.004	0.035	Up	yes	Inf	3.613	4.27E-07	Up	yes
gene-L OC432 7325	uncharacterized protein LOC4327325	-	-	-	-	-	Inf	3.508	0.003	Up	yes	Inf	1.527	0.001	Up	yes
gene-L OC433 2423	cysteine-rich repeat secretory protein 55	-	-	-	-	-	Inf	3.022	0.032	Up	yes	Inf	2.493	0.000	Up	yes
gene-L OC433 5094	syn-pimara-7,15-diene synthase	-	-	-	-	-	Inf	18.25	1.14E-08	Up	yes	Inf	0.593	0.017	Up	yes
								79.1	08				76.9	57.8		

Note: log₂FC = log₂ Fold Change. Up or down means significantly induced or suppressed.