

## **RNA-seq reveals the involvement of key genes for aerobic adaptation in rice**

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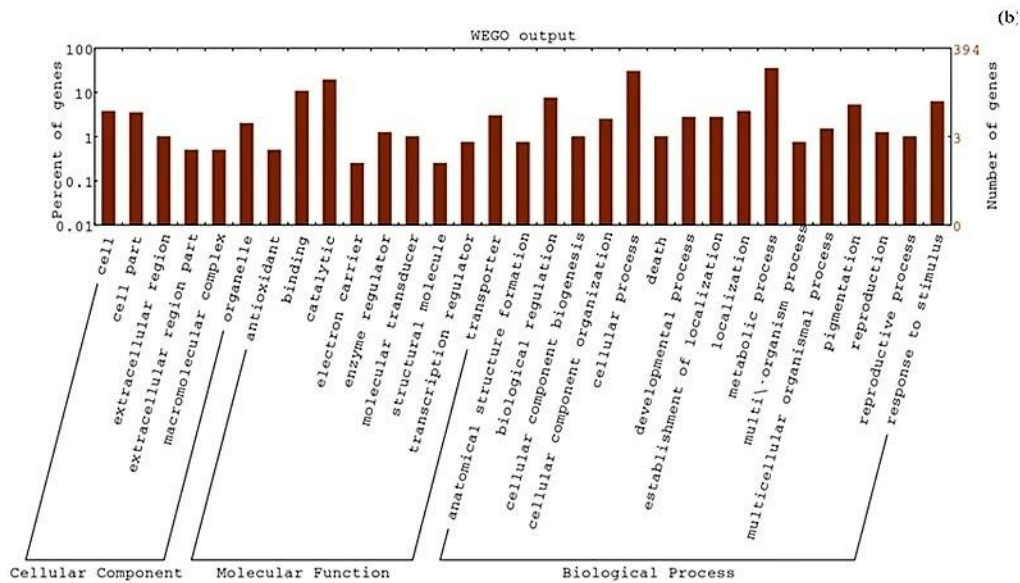
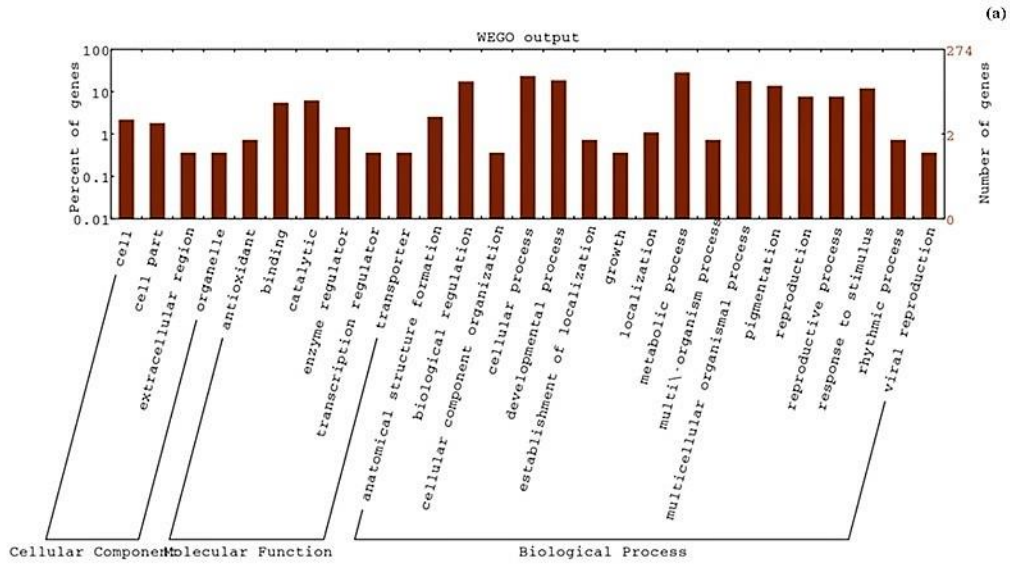
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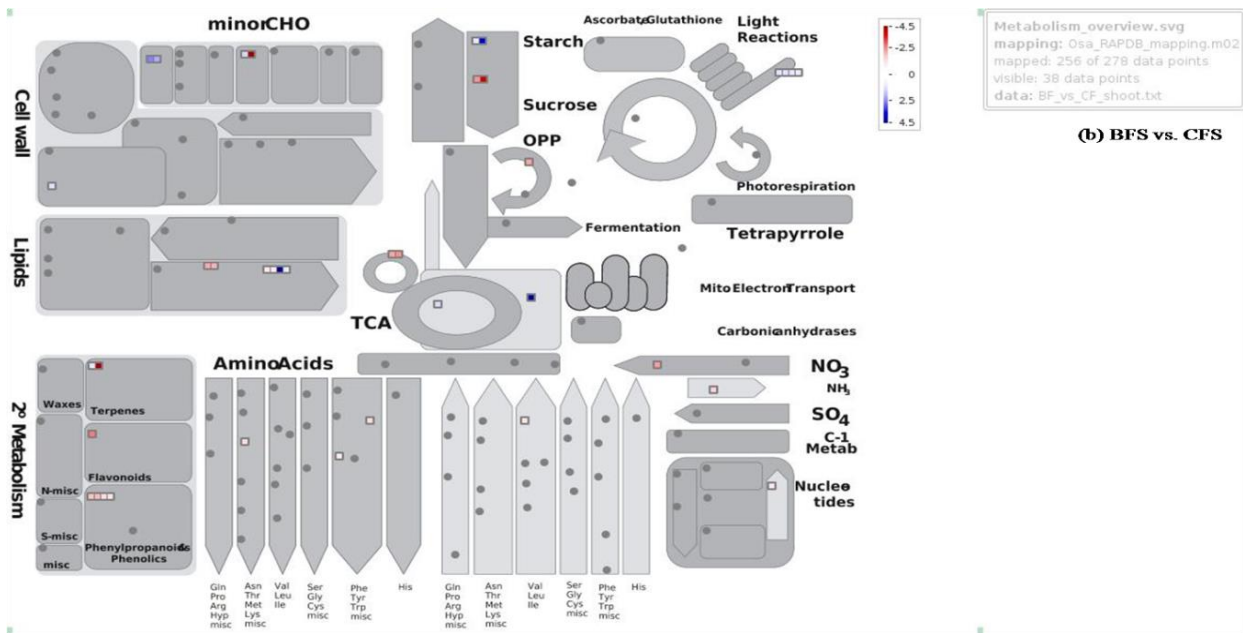
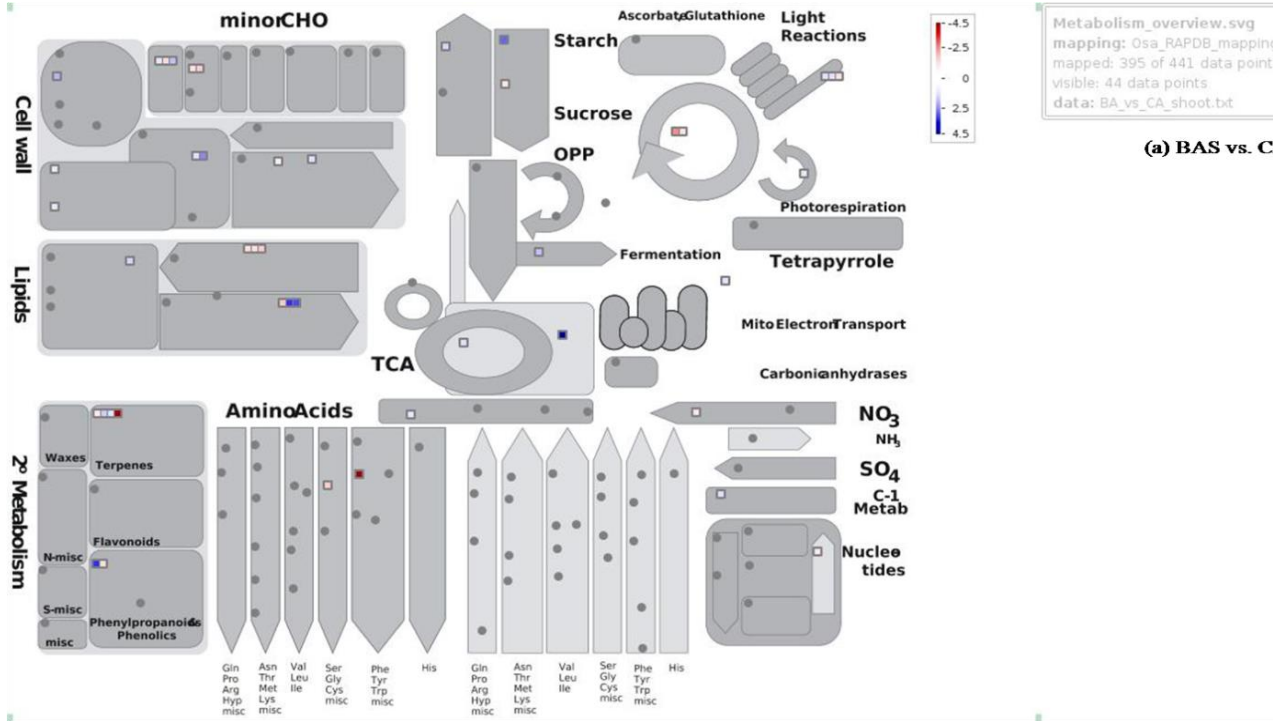
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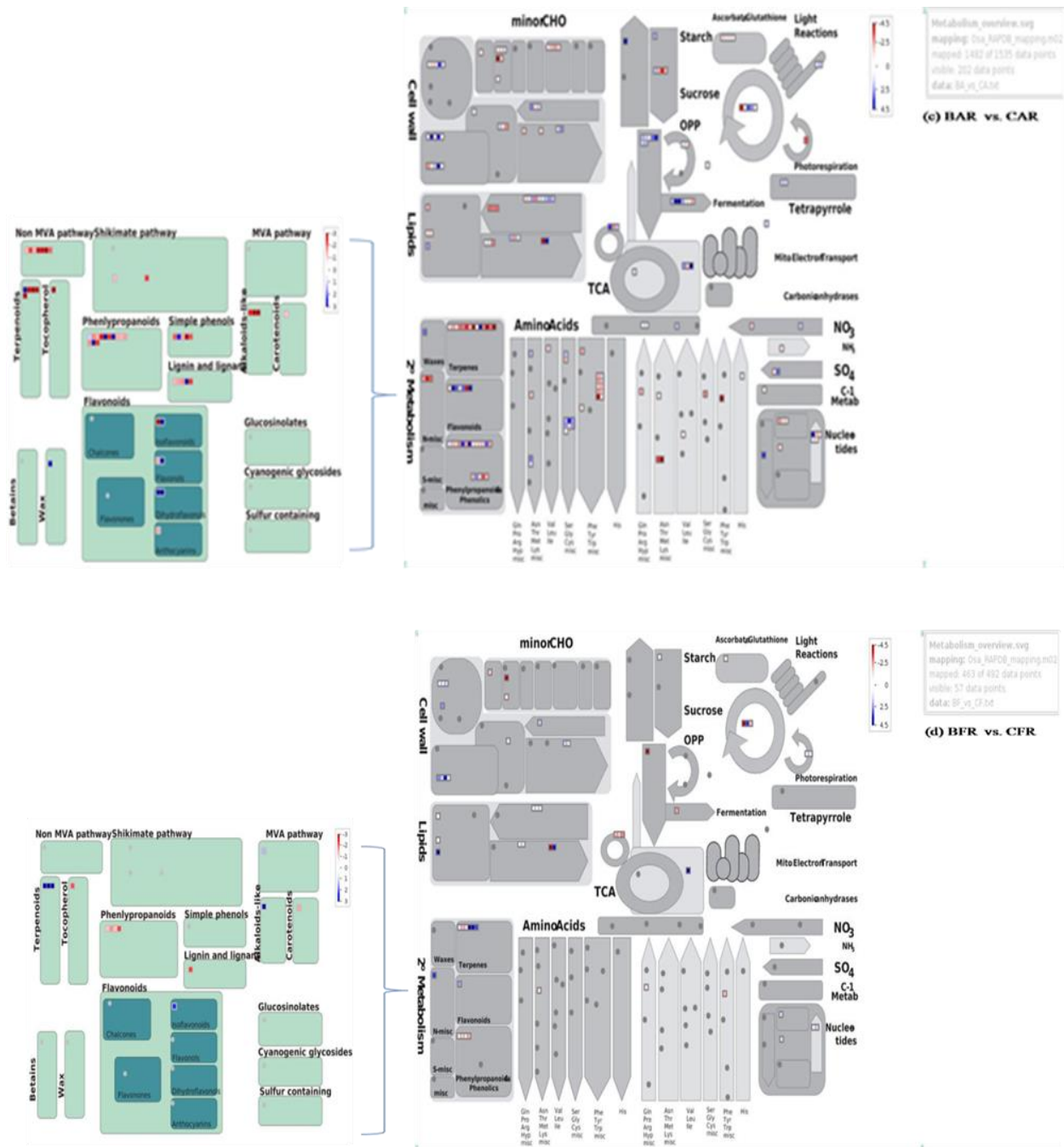
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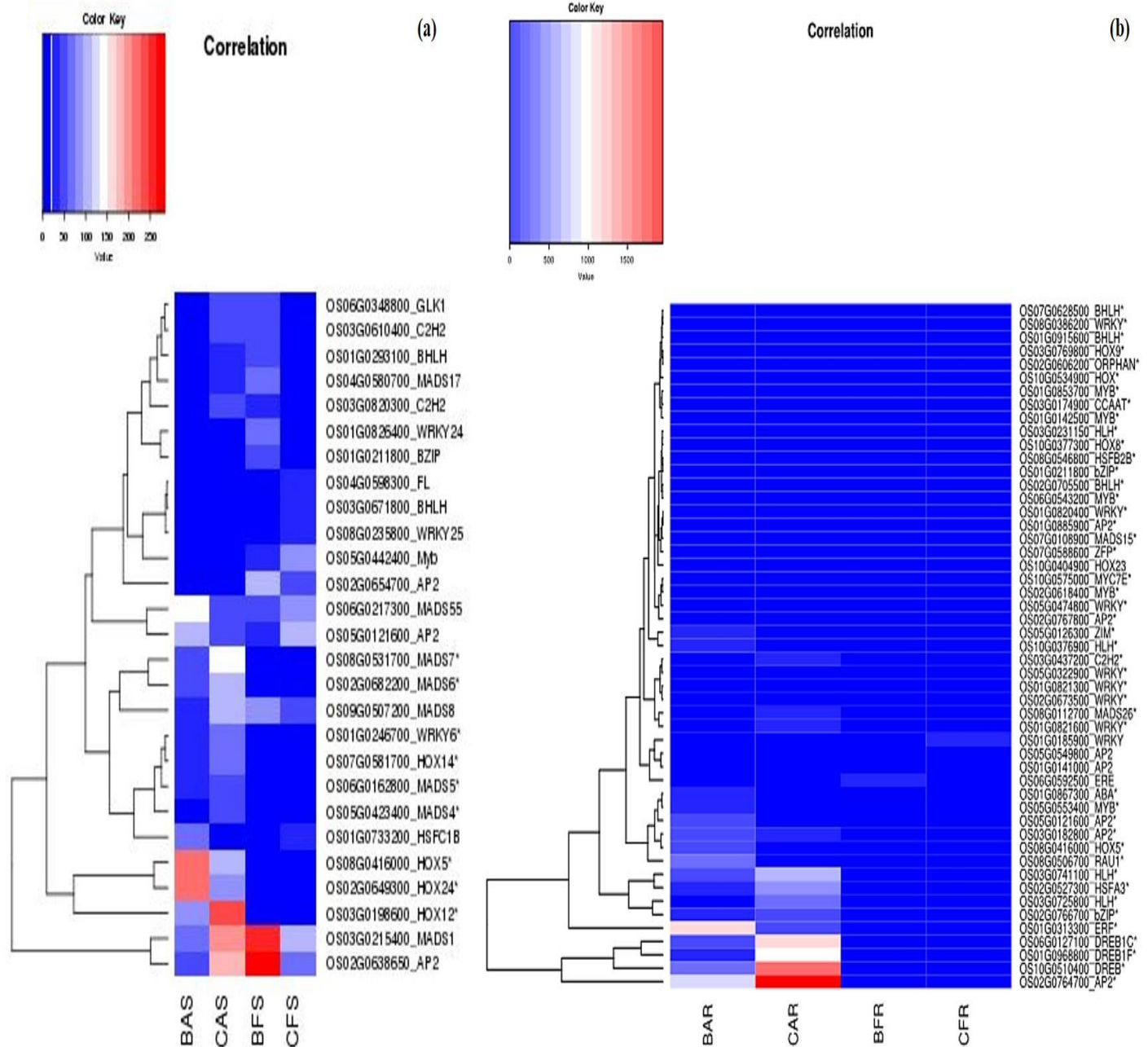
**Fig. S1. GO enrichment analysis for DETs in shoot (BAS vs. CAS) and in root (BAR vs. CAR) under aerobic conditions.** The categories of biological processes (BP) most significant followed by molecular function (MF) and cellular component GO terms in (a) shoot and (b) root are shown from top to bottom.



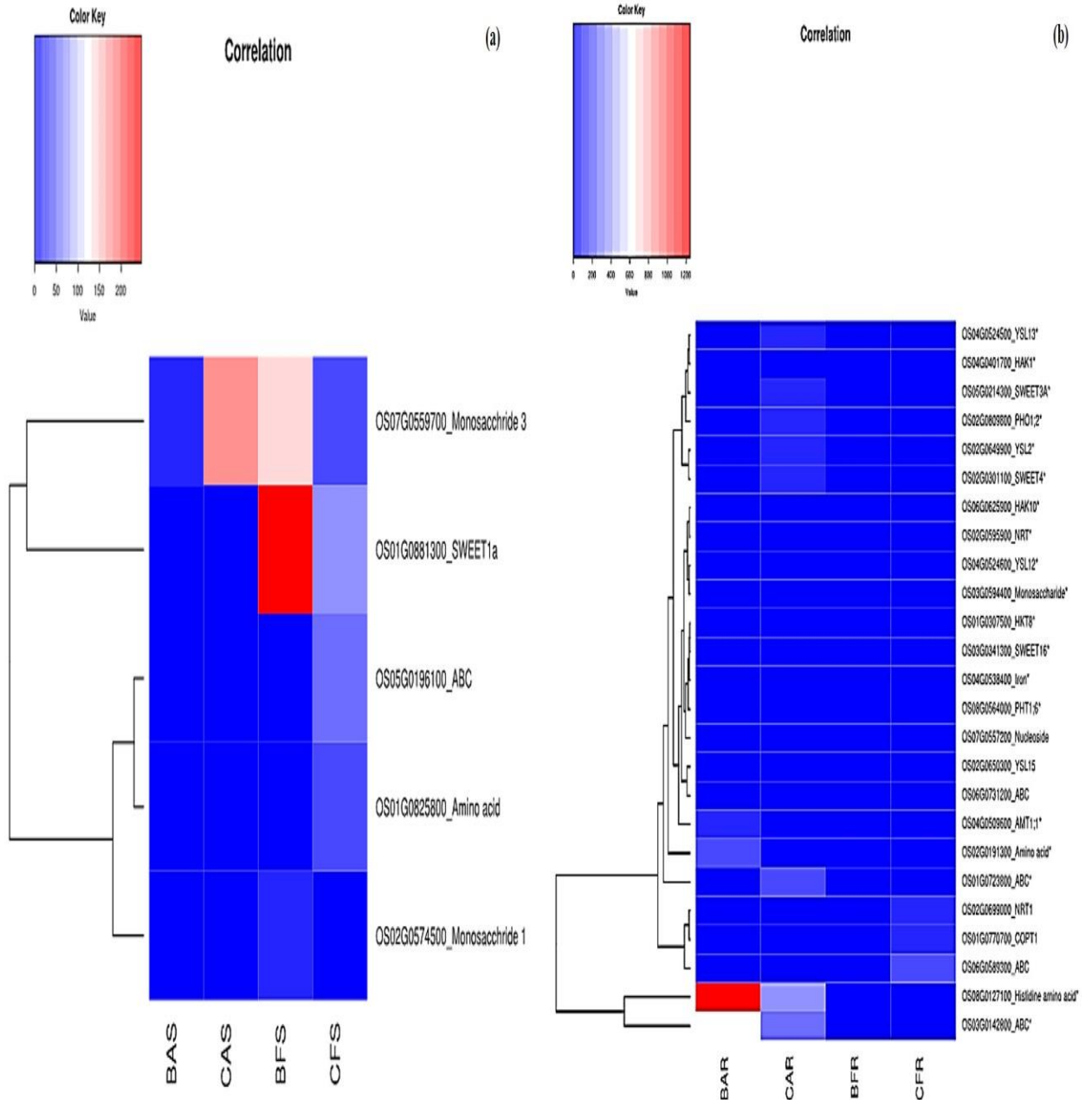


**Fig. S2. Overall metabolism operating under aerobic and anaerobic conditions in shoot (a) BAS vs. CAS, (b) BFS vs. CFS and in root (c) BAR vs. CAR, (d) BFR vs. CFR.**

Transcripts highlighted in insight involved in secondary metabolism processes are shown in the main panel in dark green, while putatively associated transcripts are shown in light green. Blue denotes up-regulation and red down-regulation.

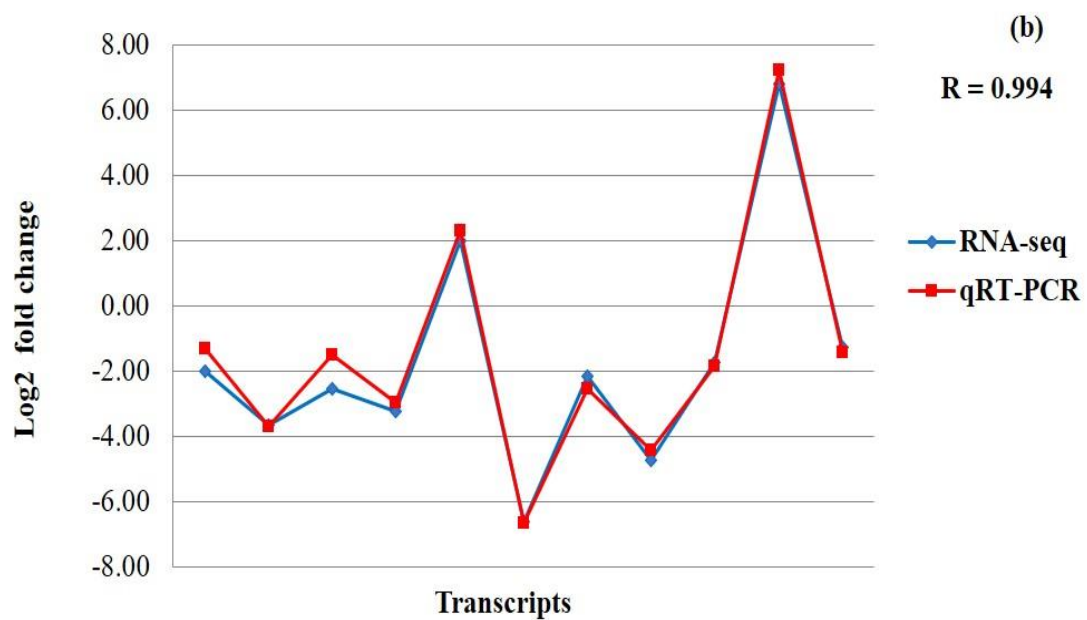
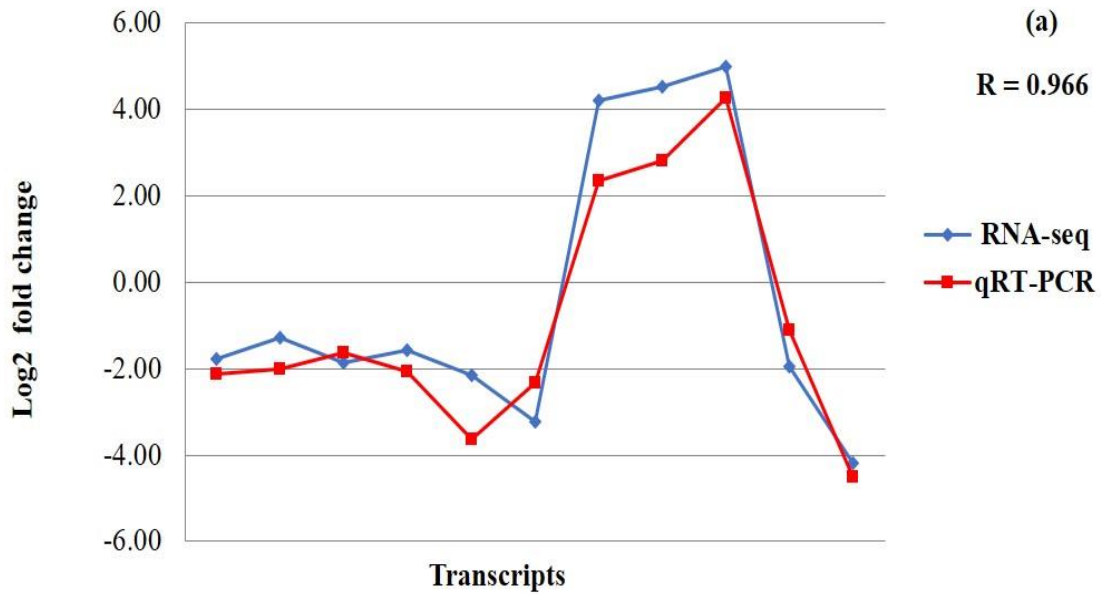


**Fig. S3. Heatmaps representation of differentially expressed TFs (mentioned on the right side) (a) in shoot (BAS, CAS, BFS, CFS) and (b) in root (BAR, CAR, BFR, CFR) under aerobic and anaerobic conditions. The color scale at the top shows expression values in read count. Red represents high levels of expression and blue indicates low levels (\* symbol denotes exclusively expressed TFs in shoot and root of aerobic adapted cultivar CR Dhan 202 under aerobic condition).**

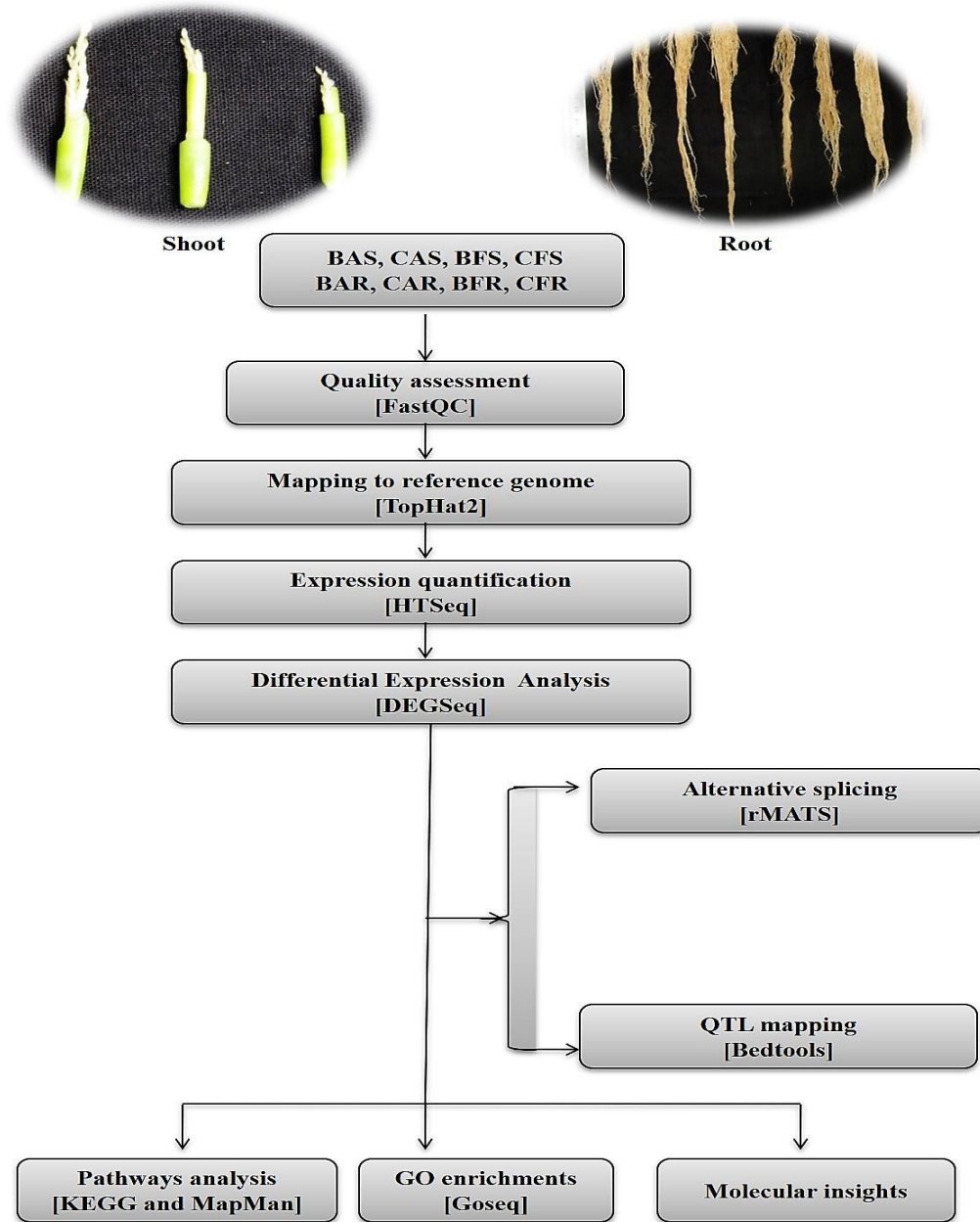


**Fig. S4. Heatmaps representation of differentially expressed transporters (mentioned on the right side) (a) in shoot (BAS, CAS, BFS, CFS) and (b) in root (BAR, CAR, BFR, CFR) under aerobic and anaerobic conditions. The color scale at the top shows expression values in read count. Red represents high levels of expression and blue indicates low levels (\* symbol denotes exclusively expressed transporters in shoot and root of aerobic adapted cultivar CR Dhan 202 under aerobic condition).**





**Fig. S5. Validation of the expression of representative transcripts from RNA-seq using qRT-PCR in (a) shoot (BAS vs. CAS) and in (b) root (BAR vs. CAR) under aerobic conditions.**



**Fig. S6. Workflow/ summary of RNA-seq data analysis in shoot and root under aerobic and anaerobic conditions.**

BAS - BPT 5204 Aerobic Shoot, CAS - CR Dhan 202 Aerobic Shoot, BAR - BPT 5204 Aerobic Root, CAR - CR Dhan 202 Aerobic Root, BFS - BPT 5204 Anaerobic Shoot, CFS - CR Dhan 202 Anaerobic Shoot, BFR - BPT 5204 Anaerobic Root and CFR - CR Dhan 202 Anaerobic Root



**Table S1. Differentially expressed transcripts (DETs) in shoot (BAS vs. CAS) (BAF vs. CFS) and root (BAR vs. CAR) (BFR vs. CFR) under aerobic and anaerobic conditions (Please see the supplementary excel file).**

**Table S2. Top ten KEGG pathway enrichment analysis of the DETs of shoot and root under aerobic and anaerobic conditions.**

Term	Database	ID	Input number	Background number	P-Value	Corrected P-Value
<b>BAS vs. CAS</b>						
Fatty acid degradation	KEGG PATHWAY	osa00071	5	38	0.0001 98021	0.0066088 24
Glyoxylate and dicarboxylate metabolism	KEGG PATHWAY	osa00630	6	63	0.0002 31889	0.0066088 24
Galactose metabolism	KEGG PATHWAY	osa00052	4	48	0.0041 34657	0.0657114 73
Tryptophan metabolism	KEGG PATHWAY	osa00380	3	24	0.0046 11331	0.0657114 73
Thiamine metabolism	KEGG PATHWAY	osa00730	2	9	0.0080 52586	0.0799734 96
Fatty acid metabolism	KEGG PATHWAY	osa01212	4	60	0.0086 32145	0.0799734 96
Metabolic pathways	KEGG PATHWAY	osa01100	30	1492	0.0098 21307	0.0799734 96
Plant-pathogen interaction	KEGG PATHWAY	osa04626	5	105	0.0127 65287	0.0898485 42
Peroxisome	KEGG PATHWAY	osa04146	4	70	0.0141 86612	0.0898485 42
<b>BFS vs. CFS</b>						
Metabolic pathways	KEGG PATHWAY	osa01100	63	1492	1.8433 4E-06	0.0011981 68
Biosynthesis of secondary metabolites	KEGG PATHWAY	osa01110	39	800	9.8663 E-06	0.0032065 48
Fatty acid degradation	KEGG PATHWAY	osa00071	6	38	0.0003 80693	0.0189637 75
alpha-Linolenic acid metabolism	KEGG PATHWAY	osa00592	6	39	0.0004 31204	0.0189637 75
Terpenoid backbone biosynthesis	KEGG PATHWAY	osa00900	6	40	0.0004 86793	0.0197759 58
Cutin, suberine and wax biosynthesis	KEGG PATHWAY	osa00073	4	16	0.0008 45517	0.0289255 91
Plant-pathogen interaction	KEGG PATHWAY	osa04626	9	105	0.0009 40201	0.0305565 34
Photosynthesis - antenna proteins	KEGG PATHWAY	osa00196	4	17	0.0010 26452	0.0317711 34
Thiamine metabolism	KEGG PATHWAY	osa00730	3	9	0.0020 04785	0.0328503 76
<b>BAR vs. CAR</b>						
Biosynthesis of secondary metabolites	KEGG PATHWAY	osa01110	71	800	8.0196 4E-08	7.29787E- 06
Metabolic pathways	KEGG	osa01100	103	1492	8.5882	0.0003101

	PATHWAY				4E-06	02
Glycolysis / Gluconeogenesis	KEGG PATHWAY	osa00010	19	125	1.02231E-05	0.000310102
Biosynthesis of amino acids	KEGG PATHWAY	osa01230	22	193	0.000115963	0.002638161
Phenylalanine, tyrosine and tryptophan biosynthesis	KEGG PATHWAY	osa00400	8	39	0.00070072	0.012753099
Phenylpropanoid biosynthesis	KEGG PATHWAY	osa00940	14	113	0.000935942	0.014195117
Carbon metabolism	KEGG PATHWAY	osa01200	22	235	0.001348053	0.017524693
Arginine and proline metabolism	KEGG PATHWAY	osa00330	7	35	0.001706147	0.019407426
Starch and sucrose metabolism	KEGG PATHWAY	osa00500	16	156	0.002553126	0.025814936
<b>BFR vs. CFR</b>						
Arachidonic acid metabolism	KEGG PATHWAY	osa00590	2	10	0.010699255	0.159986991
Plant hormone signal transduction	KEGG PATHWAY	osa04075	7	177	0.011914325	0.159986991
Biosynthesis of secondary metabolites	KEGG PATHWAY	osa01110	19	800	0.014062773	0.159986991
Pyruvate metabolism	KEGG PATHWAY	osa00620	4	72	0.018745371	0.159986991
Phenylpropanoid biosynthesis	KEGG PATHWAY	osa00940	5	113	0.021012259	0.159986991
Linoleic acid metabolism	KEGG PATHWAY	osa00591	2	15	0.021103488	0.159986991
Carbon fixation in photosynthetic organisms	KEGG PATHWAY	osa00710	4	75	0.021289591	0.159986991
Starch and sucrose metabolism	KEGG PATHWAY	osa00500	6	156	0.021693151	0.159986991
Glycolysis / Gluconeogenesis	KEGG PATHWAY	osa00010	5	125	0.030287904	0.1623538

**Table S3. Details of uniquely expressed root related transcripts under aerobic conditions in the root of two cultivars.**

<b>Phenotype</b>	<b>Gene id</b>	<b>BAR read count</b>	<b>CAR read count</b>	<b>Gene name</b>	<b>Gene description</b>
<b>Root development related genes</b>					
Crown root formation	Os01G0149800	52.8	490.9	<i>MT2A</i>	Metallothionein-like protein 2A
	Os05G0111300	150.1	657.7	<i>MT2C</i>	Metallothionein-like protein 2C
	Os01G0974200	1144	3471.7	-	Metallothionein-like protein 2B
<b>Genes related to physiological functions of root</b>					
Root hair development	Os10G0555900	6.5	34.6	<i>EXPB3</i>	Expansin-B3
<b>Genes related to physiological functions of root</b>					
P uptake	Os08G0564000	1.6	42.6	<i>PHT1;6</i>	Inorganic phosphate transporter 1-6
K and Na uptake	Os01G0307500	1.9	32.5	<i>HKT8</i>	Cation transporter HKT8
<b>Genes related to utilization of water and micronutrients</b>					
Water acquisition	Os04G0521100	2.6	22.5	<i>PIP2;3</i>	Probable aquaporin PIP2-3

**Table S4. Details of alternative splicing (AS) events of differentially expressed transcripts (DETs) in shoot (BAS vs. CAS, BFS vs. CFS) and in root (BAR vs. CAR, BFR vs. CFR) under aerobic and anaerobic conditions.**

Alternative Splicing (AS) Event	In shoot		In root	
	Aerobic (BAS vs. CAS)	Anaerobic (BFS vs. CFS)	Aerobic (BAR vs. CAR)	Anaerobic (BFR vs. CFR)
Retained intron (RI)	380	370	299	297
Alternative 3' splice site (A3'SS)	170	159	138	139
Alternative 5' splice site (A5'SS)	61	63	61	49
Skipped exon (SE)	47	46	42	42
Mutual exclusive exons (MXE)	1	1	1	0

**Table S5. Details of primers used for shoot (a) and root (b) RNA-seq data validation through qRT-PCR.**

S. No	Gene symbol	Gene description	Locus information	Primer sequence 5' to 3' (forward/reverse)	Amplicon length (bp)	Tm (0C)
<b>(a) For shoot</b>						
1	<i>OsBURP3</i>	BURP domain-containing protein 3	LOC_Os01g53240	CCAAGCGCAGCTCAATCTTTGC TGGCTTCCTACTGAAGCGATCAAC	110	62
2	<i>OsCML15</i>	Probable calcium-binding protein CML15	LOC_Os05g31620	AAGGTGAGGGCGTTCTTCTC ACCAGTCCACGTTTCATCGC	91	62
3	<i>OsGLK1</i>	Probable transcription factor GLK1	LOC_Os06g24070	GCTTCAACTCCAGGTTCAACCA TC AGTCCAAGAGGCAAAGGCAAC C	93	62
4	<i>OsHOX12</i>	Homeobox-leucine zipper protein HOX12	LOC_Os03g10210	AGCAAGCTCATGGAGGAGGAG TTC TTCAGCTTCAGCAACTCGGTCT CG	131	62
5	<i>OsMADS8</i>	MADS-box transcription factor 8	LOC_Os09g32948	TCACTGATCTCCAGAGGAGGGA AC CCACACTTGTCATGCAACTGG	98	62
6	<i>OsMT2A</i>	Metallothionein-like protein 2A	LOC_Os01g05650	TGCTCGTGTGTTGTGCGATCAG TCTCCGGGTACATCTTGCATCC TC	149	62
7	<i>OsPIP1;3</i>	Aquaporin PIP 1;3	LOC_Os02g57720	ACCGAGTTGATGCTGCAAGATG ATCACCAGACGGTAATAACAC ACG	73	62
8	<i>OsRAB21</i>	Water stress-inducible protein Rab21	LOC_Os11g26790	AGCTCAAGCTCGTCTGAGGATG GGGAGCTTCTCCTTGATCTTCT CC	80	62
9	<i>OsRAB16B</i>	Dehydrin Rab16B	LOC_Os11g26780	TTCGCACGTGTTGGGTTGTACC ACACACACACCATACAAAGCTT GC	65	62
10	<i>OsTPR</i>	TPR Domain containing protein	LOC_Os03g19560	ACAAGCTACCCAATGCAATCCC GACTTGCTGGACTGCTTTCTC	122	62
11	<i>Putative</i>	Putative uncharacterized protein	LOC_Os05g11810	TCGTGGGAAGTTGAAGCCACTT G TCTCCCGTTTCGTTAGAACCCTTG C	141	62
<b>(a) For root</b>						
1	<i>HLH</i>	Helix-loop-helix DNA-binding domain	LOC_Os03g51580	TCCCAGAACCAGGAACACATCC CGACGCCTTGTCATCTTCTTG	114	62



		containing protein				
2	<i>OsBK11</i>	Brassinosteroid insensitive1-associated receptor kinase 1	LOC_Os11g31540	CATTAGCACCTGCGAAATCTGAG TCGTCAGGCTGCCCAAAGATTG	80	62
3	<i>OsDREB1F</i>	Dehydration-responsive element-binding protein 1F	LOC_Os01g73770	TTCGTCCTCGTCGGTGT GCGTCTCCCGGAATTTCTT	110	62
4	<i>OsMT2A</i>	Metallothionein-like protein 2A	LOC_Os01g05650	TGCTCGTGTGTTGTGCGATCAG TCTCCGGGTACATCTTGCATCC TC	149	62
5	<i>OsNAC71</i>	NAC domain-containing protein 71	LOC_Os11g08210	TTAACGGAACGTGACGCCTCTG ACGATCGACCTGACAGTGGTTA G	63	62
6	<i>OsNAS1</i>	Nicotianamine synthase 1	LOC_Os03g19427	TGCTGCATTTGCGAAGCTAAGG ACTGTACGTCCCAAACAACACG	76	62
7	<i>OsPHO1;2</i>	Phosphate transporter PHO1;2	LOC_Os02g56510	TAAGCCACAGCAGCCCAGAAAC C AGTGCCCGTGAACAAACCTACG	64	62
8	<i>OsPHT1;6</i>	Inorganic phosphate transporter 1;6	LOC_Os08g45000	TGCAAAGTGTACTGTAGCGCTG TC TGGCGGTTTCTTCGATCGATCT TC	124	62
9	<i>OsPIP1;3</i>	Aquaporin PIP 1;3	LOC_Os02g57720	ACCGAGTTGATGCTGCAAGATG ATCACCAGACGGTAATAACAC ACG	73	62
10	<i>OsRAB21</i>	Water stress-inducible protein Rab21	LOC_Os11g26790	AGCTCAAGCTCGTCTGAGGATG GGGAGCTTCTCCTTGATCTTCT CC	80	62
11	<i>OsYSL2</i>	Metal-nicotianamine transporter YSL2	LOC_Os02g43370	ACTTGCTTCCACTCAGAAAGGC GTTGCAGTCCCACTTGGATAGG	67	62