Pathway Analysis of the Differentially Expressed Genes in Oryza Sativa Exposed to the Implantation of Low-Energy Nitrogen Ion

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***Abstract*—In order to investigate the overall characteristics of the transcript profiles in rice cell underlying the radiation of the ion beam. We used the Agilent Rice Oligo Microarray （4×44K） Genome Array to investigate the differentially expressed genes in rice responding to low-energy ion-implantation. Rice seeds were implanted by the nitrogen ion beam and their vigor index was investigated at ten days after germination. The measuring of the vigor index showed that lower-dose implantation of the nitrogen ion beam (6×1017 N+/cm2) enhanced the vigor index of the rice seedlings and the higher-dose implantation (9×1017 N+/cm2) damaged the rice seedlings because of the weaker vigor index. The analysis of the Genechip Array showed that there were 982 genes expressed differentially (fold change＞2 and P value＜0.05), including 429 up-regulated genes and 553 down-regulated genes underlying the dose3:6×1017 N+/cm2. 15 out of the 429 up-regulated genes were involved in 22 pathways including carbohydrate metabolism, translation, biosynthesis of secondary metabolites, lipid metabolism, energy metabolism, replication and repair, nucleotide metabolism, signal transduction, folding, sorting and degradation. 30 out of the 553 down-regulated transcripts were involved in 48 pathways. Our analysis revealed that the differentially expressed genes involving important pathways were compatible with the distinct cellular events in response to stress of ion implantation. It supplied the first comprehensive and comparative molecular information for further understanding the mechanism in plant cell exposing to the low-energy ion beam.**

***Index Terms*—Ion beam implantation, Pathway analysis, Rice, Transcriptome.**

I. INTRODUCTION

Chinese scientists discovered the biological effect of ion beam implantation in 1980s, and applied this method to the mutation breeding. As a new resource of mutagens, low-energy ion beam implantation is characterized as limited physiological damages, wide mutation spectrum, and high mutation frequency in comparison with the other mutagens [1].

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The mutagenic effect of low-energy ion beam implantation on cereal seeds has been substantiated by many studies in the past thirty years. Many of these studies focus on the physical factors on the biological effect. At a definite amount of energy into organism, a process including ion mass deposition, energy deposition and charges exchange must have taken place. The implanted ions punch atoms of molecules in double-stranded DNA chains and force them to shift from their original position. The shifted atoms might interact with other elements in the DNA molecule and further form new molecules, or the shifted atoms just leaved and lots of empty remain at their original position. The former leads to genetic effect such as base substitutions, the latter causes deletions and insertions of a single base or a small DNA fragment, even chromosome breakage and translocation [2]. Low-energy ion can reach nuclei and causes damages in DNA molecule, thus inducing mutations when the DNA repair fails [3]. It is substantiated that the biological effects induced by ion beam implantation were greatly different with these by physical radiations such as X-rays [4]. For example, vigor index of the plant seedlings with the seedlings implanted by ion beam displayed a "saddle-type" change with the upward dosage gradient. Up to now the exact molecular mechanism of the ion-implantation biological effects on cereals has not been fully understood, especially the overall characteristics of expression profiles associated with these puzzling biological effects have not been reported until today. A comprehensive analysis of transcriptome profiles of seedlings with exposure to the ion implantation is important to understand the conserved and diverse mechanism of the biological effects.

Rice has been used as an excellent model plant in molecular genetics after Arabidopsis, because of its relatively smaller genome and the completion of the genome sequence. Ion-beam biotechnology had been fully applied in rice mutation breeding. In order to understand the molecular mechanism of the ion-implantation biological effects, we analyzed transcriptome features of rice exposing to the nitrogen ion beam implantation, using the agilent rice oligo microarray （4×44K） genome array involving more 40,000 EST. These results shed light on the overall characteristics of expression profiles [5] associated with genes responding to the stress of the ion beam radiation and the key components of the regulatory network regulating the processes, especially the pathway involved in this response.

II. Materials and Methods

A. Plant materials

Rice cultivar Zhonghua 10 *(Oryza sativa L. ssp. japonica*) was used for this study. Completed buds leaves were collected after the seeds being incubated 96-hours in a dark climate chamber at 28℃. All seeds were planted on sterile medium with 0.6% agar only.

B. Implantation of the low-energy N^+ ion beam and vigor index

The seeds were implanted by the low-energy (40 Kev) N^+ ion beam (generated by a machine: UIL.0.512, TNV. Russia) in the dosages: 1×10^{17} N^+/cm^2, 3×10^{17} N^+/cm^2, 6×10^{17} N^+/cm^2, 9×10^{17} N^+/cm^2.Three replications were done at each dosages, two hundred seeds were implanted each replicates. The untreated seeds were as the controls.

The germination percentage was investigated after the seeds germinated

Germination percentage=Number of the seedlings/total seeds*100%

Vigor index =Drought weight * Germination percentage

C. RNA extraction

Total RNA was extracted from uniform thirty individual buds in each replicate, which were planted for 96 hours, using RNA plant reagents (Tiangen Biotech) and purified by use of the RNeasy Plant Kit (Qiagen) according to the manufacturer's instruction. The yield and purity of RNA were determined spectrophotometrically (Nanodrop ND1000).

D. Agilent GeneChip hybridization and data analysis

The Agilent GeneChip hybridization and data analysis were carried out by the ShanghaiBio Company Ltd., including the procedures for cDNA and cRNA synthesis, cRNA Cy3 fluorescence labeling (GE healthcare PA13105), hybridization (Agilent G2545A), washing, scanning (Agilent G2565BA Microarray Scanner System), data collection and normalization.

E. Evaluation of the data credibility

The quantitative variation of ten housekeeping genes were used to assess the data credibility of the genechip data between the treated samples and the controls, these housekeeping genes displayed highly uniform expression in living organisms during various phases of development and under different environmental conditions and were frequently used as the validated internal control in quantitative real-time PCR.

F. Bioinformatics analyses

Fold Change, t-Test, significant analysis of microarray, one-way ANOVA, principal component Analysis, heat map, GO enrichment analysis and pathway enrichment analysis were analyzed by use of SBC Analysis System (a web server, http://sas.ebioserivce.com).

III. Results and Disscussion

A. Vigor index of the rice implanted by the low-dose nitrogen ion beam

The vigor index of one hundred seedlings of was investigated after the seeds planted 10 days in every experiment replicate. The results showed that smaller-influx implantation of the nitrogen ion beam (6×10^{17} N^+/cm^2) enhanced the vigor index ($P<0.05$) of the rice seedlings and the larger-influx implantation (9×10^{17} N^+/cm^2) damaged the rice seedlings because of the smaller vigor index than the controls ($P<0.05$) (Table Ⅰ). It was interested that most of the improved corn variety derived from the ion-beam technology were induced underlying the smaller ion influx which the vigor index of the treated material were superior to the control [6]. However, the low-energy ion hardly penetrated seed coat that most of the seeds [7]. So the low-energy ion hardly damaged the DNA in the cell nuclear of the grain seeds with exposure to the low-energy ion implantation. Here, it suggested that the secondary rays derived from the implanted nitrogen induce the cell response to comprehensive stress involving in ionizing radiation, drought tolerance, and salt tolerance. The vitality of the rice seedlings were enhanced because the response to comprehensive stress through regulating the divergence of the genes expression related to some metabolism.

TABLE I: Vigor Index of the Rice Seedlings

Samples	Germination percentage (Mean%±STD)	Vigor index (Me±STD)	P-value T-test to vigor index
Control	81.85±2.31	9.55±1.69	
Dose1	84.81±2.21	12.14±0.8	0.074
Dose2	86.67±1.11	12.14±1.02	0.085
Dose3	88.89±0.00	14.52±1.36	0.017
Dose4	51±7.2	5.29±0.99	0.02

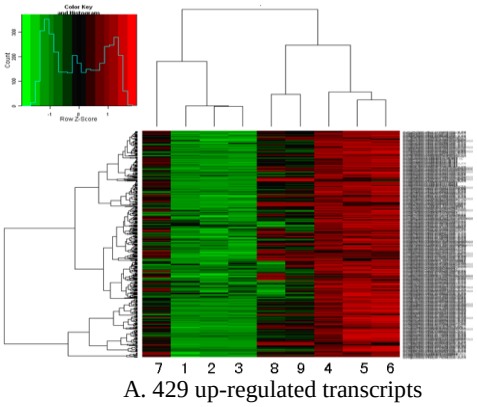
Dose1, 1×10^{17} N^+/cm^2; Dose 2, 3×10^{17} N^+/cm^2; Dose 3, 6×10^{17} N^+/cm^2; Dose 4, 9×10^{17} N^+/cm^2; STD, standard deviation

B. The differentially expressed genes profiles responding to the stress of the ion beam radiation

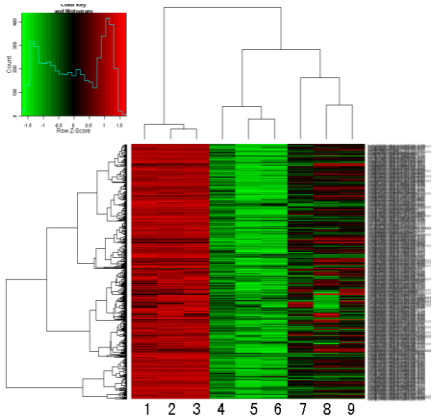
In order to understand which genes contributed to **t**he enhanced vitality, the RNAs samples from the seedlings exposing to the implantation of ion beam at dose 3 **(**6×10^{17} N^+/cm^2**),** dose 2 **(**3×10^{17} N^+/cm^2**)** and the controls were probed by the Agilent Rice Oligo Microarray (4×44K) Genome Array including more 40,000 EST. Three independent biological replicates were performed for each dosage. Here, we focus on expression profiles under the dose 3 for the significant variation of the vigor index (P value $<$ 0.05). There are 982 differentially expressed transcripts (fold change >2 and P value <0.05) including 429 up-regulated transcripts (Fig1-A) and 553 down-regulated transcripts (Fig1-B).

C. Evaluation of the data credibility of the genechip

Ten housekeeping genes (Table Ⅱ) that were frequently used as the validated internal control in quantitative real-time PCR were chosen to assess the data credibility of the genechip. These data are from the Genechip Array to samples implanted by the dose 3 ($6\times10^{17}N^+/cm^2$), and the results showed that Fold Change of these ten genes are close to 1, namely these genes displayed highly uniform expression in seedlings of the controls and treated materials. So it suggested that the data from the Genechip Array to samples are vailable.



A. 429 up-regulated transcripts



B. 553 down-regulated transcripts

Fig. 1. Heatmap of the transcripts expressed differentially

Samples 1, 2 and 3 are the three independent biological replicates of the controls respectively, samples 4, 5 and 6 are the three independent biological replicates of the samples treated by dose 3 ($6\times10^{17}N^{+}/cm^{2}$), samples 7, 8 and 9 are the three independent biological replicates of the samples treated by dose 2 ($3\times10^{17}N^{+}/cm^{2}$). The red represent the higher-level expression and the green represent the lower-level expression

TABLE II. EXPRESSION OF THE TEN HOUSEKEEPING GENES

Short description of the housekeeping genes	Probe number in genechip	Relative expression Mean+STD
Actin 1	10	1.01±0.14
Ubiquitin-conjugating enzyme E2	15	0.95±0.14
Eukaryotic elongation factor 1-alpha	20	1.11±0.09
Histone H2A	16	0.87±0.23
Glyceraldehyde-3-phosphate dehydrogenase	18	1.14±0.19
Beta-tubulin	2	1.01±0.03
Eukaryotic initiation factor 4a	10	1.10±0.17
Ubiquitin	7	1.07±0.27
Cyclophilin	10	0.8±50.10
Sulfite reductase	3	0.99±0.11

STD: standard deviation

D. Pathway enrichment analysis of the up-regulated genes responding to the ion-beam

15 out of the 429 up-regulated transcripts were involved in 22 pathways (Table Ⅲ) including carbohydrate metabolism, translation, biosynthesis of secondary metabolites, lipid metabolism, energy metabolism, replication and repair, nucleotide metabolism, signal transduction, folding, sorting and degradation. It is interested that the phosphatidylinositol signaling system were up-regulated because the signal transduction was helpful to understand the signals rated to the response to the implantation of the ion beam.

7 out of these 15 transcripts were associated with more than two interrelated pathways (Table Ⅳ). Especially, the hybridization signal of the anthocyanidin reductase transcripts in flavonoid biosynthesis (enrichment percent: 10%) and ATP-dependent DNA helicase, 70 kDa subunit family protein transcript (Non-homologous end-joining) in DNA replication (12.50%) were not detected in the total RNA of the control samples, but were detected remarkably in the samples implanted by ion beam. Anthocyanidin reductase (ANR), encoded by the BANYULS gene, is a newly discovered enzyme of the flavonoid pathway involved in the biosynthesis of condensed tannins. Catechins are not only the most important components in tea flavor, but also possess a lot of physiological functions, such as antioxidant activity, antimutagenic and anticarcinogenic potential, anti-cardiovascular diseases, anti-ultraviolet radiation and so on [8]-[9]. It suggested that the flavonoid pathway was related to the rice responding to the implantation of the nitrogen ion because the transcripts of anthocyanidin reductase express preferentially under the ion-beam radiation.

The minichromosome maintenance (MCM) proteins are essential for DNA replication in eukaryotes. Thus far, all eukaryotes have been shown to contain six highly related MCMs that apparently function together in DNA replication [10]. The ATP-dependent DNA helicase (EC:3.6.1) is involved in genome maintenance and help the DNA replication [11]. The bigger vigor index of the rice seedlings with exposure to dose 3 **(**6×10^{17} N^{+}/cm^{2}**)** suggested that the DNA in the cell replicate in shorter cycle, the result of the ATP-dependent DNA helicase confirmed the physiology on the molecular level.

E. Pathway enrichment analysis of the down-regulated genes responding to the ion-beam

30 out of the 553 down-regulated genes were involved in 48 pathways (Table Ⅴ,) including carbohydrate metabolism, translation, Metabolism of Cofactors and Vitamins, behavior, Amino Acid Metabolism, biosynthesis of secondary metabolites, lipid metabolism, energy metabolism, replication，repair and so on. It is interested that the RecA bacterial DNA recombination protein family protein were down-regulated.

14 out of these 30 genes were associated with more than two interrelated pathways (Table Ⅵ, showed partially). Os04g0518400 (Phenylalanine ammonia-lyase 2 (PAL; EC 4.3.1.5; dwon-regulated 3.3 folds; p value=0.005) were involved in 7 pathways, Os07g0446800 (Hexokinase; dwon-regulated 2.8 folds; p value=0.006) were involved in 12 pathways, and Os02g0730000 (Mitochondrial aldehyde dehydrogenase ALDH2a; dwon-regulated 2.2 folds; p value=0.019) were involved in 13 pathways.

Phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) is the enzyme at the entry-point of the phenylpropanoid pathway. PAL activity and the activation of PAL under stress

conditions have been considered part of a defence mechanism operating in stress-afflicted cells. PAL is encoded by a small multigene family of 2–6 members. Individual members of the PAL gene family are differentially expressed in plant tissues as well as in the response to different stress conditions (review, Maria T. Sanchez-Ballesta et al)[12]. PAL is usually recognized as a marker of environmental stress in differing plant tissues. We found that other members of PAL multigene family were up-regulated or no-differential expressed by the analysis of the genechip, only this probe (Os04g0518400 (Phenylalanine ammonia-lyase 2) was down-regulated. It suggests that phenylalanine ammonia-lyase 2 is negatively related to the ion beam implantation. On the other hand, Heat shock treatments delay the increase in phenylalanine ammonia-lyase activity by altering its expression (*Lactuca sativa*) tissue [13]. Implantation of the ion beam can induce a high temperature at 200℃, so it is interested that phenylalanine ammonia-lyase 2 responds the low-energy ion radiation in differential mechanism with other members in PAL family, this findings is corresponding to the higher vigor index of the rice with exposure to the low-energy N^+ ion beam implantation at the dose 6×10^{17} N^+/cm^2.

Acetaldehyde is harmful to cells because of its tendency to form adducts with protein and DNA. Cells possess mechanisms to metabolize acetaldehyde. In human, ALDH (aldehyde: NAD (P)T oxidoreductase, EC1.2.1.3) is encoded by a large family of genes, one of which encodes the mitochondrial ALDH2. ALDH2 plays an important role in the metabolism of ethanol-derived acetaldehyde. In plants, mitochondrial ALDH genes have been identified in several species, including maize, rice, barley, tobacco and Arabidopsis [14]. Mitochondrial ALDH is encoded by at least two genes in rice. It is found that the *Aldh2a* mRNA was present at high levels in leaves of dark-grown seedlings, mature leaf sheaths, and panicles [15]. Low-energy ion exposure is harmful to the rice seedlings in general, but the low-dose ion exposure enhanced the vigor index, so it should be little accumulation of the harmful acetaldehyde in rice cell under the ion implantation because of the down-regulated *Aldh2a*.

IV. Conclusions

The differentially expressed genes,involving important pathways, are compatible with the distinct cellular events in response to implantation of low-energy ion beam.The up-regulated genes involving in signal transduction, energy, biosynthesis of secondary, replication, repair and translation, are all related to the cell division and cell growth that contributed to the enhanced vigor index. The down-regulated genes, involving in the phenylalanine ammonia-lyase 2 (PAL;EC4.3.1.5) and mitochondrial aldehyde dehydrogenase (ALDH2),are responding to the stress. These findings suggested that the rice seedlings are not damaged seriously by implantation of the low-energy (40Kev) N^+ ion, but enhanced the cell growth. Our analysis of the genome-wide gene expression profiles of the rice seedlings revealed dynamic characteristics of transcriptome in plant cell underlying the implantation of the low-energy ion beam, and would led to the identification of the genes responding the ionizing radiation.

TABLE III: Pathways to Up-Regulated Gegens

Pathway Name	Hits	Percent	Short description of the hit transcripts	Category	PathwayId
Amino sugar and nucleotide sugar metabolism	1	1.28%	Endochitinase a precursor	Carbohydrate metabolism	osa00520
Aminoacyl-tRNA biosynthesis	2	4.00%	Leucyl-tRNA synthetase aminoacyl-t-RNA synthetase	Translation	osa00970
Ascorbate and aldarate metabolism	1	4.17%	Thylakoid-bound ascorbate peroxidase	Carbohydrate metabolism	osa00053
Biosynthesis of phenylpropanoids	1	0.60%	Anthocyanidin reductase	Biosynthesis of secondary metabolites	osa01061
Flavonoid biosynthesis	1	10.00%	Anthocyanidin reductase	Biosynthesis of secondary metabolites	osa00941
Glutathione metabolism	1	2.22%	Thylakoid-bound ascorbate peroxidase	Metabolism of other amino acids	osa00480
Glycerolipid metabolism	1	3.23%	Phospholipid/glycerol acyltransferase family protein.	Lipid metabolism	osa00561
Glycerophospholipid metabolism	2	4.35%	CDP-alcohol phosphatidyltransferase and phospholipid/glycerol acyltransferase family protein.	Lipid metabolism	osa00564
Glyoxylate and dicarboxylate metabolism	1	3.03%	Glycolate oxidase	Carbohydrate metabolism	osa00630
Inositol phosphate metabolism	1	3.57%	CDP-alcohol phosphatidyltransferase	Carbohydrate metabolism	osa00562
Methanemetabolism	1	2.94%	Peroxiredoxin	Energy metabolism	osa00680
Nitrogen metabolism	1	3.45%	Ferredoxin-dependent glutamate synthase, chloroplast	Energy metabolism	osa00910
Non-homologous end-joining	1	12.50%	ATP-dependent DNA helicase, 70 kDa subunit family protein.	Replication and repair	osa03450
Oxidative phosphorylation	1	0.83%	Proton pump 3 or ATPase 3, plasma membrane-type	Energy metabolism	osa00190

Peroxisome	1	1.96%	Short chain alpha- hydroxy acid oxidase	Carbohydrate metabolism	osa04146
Phenylalanine metabolism	1	2.94%	Peroxiredoxin	Energy metabolism	osa00360
Phenylpropanoid biosynthesis	1	2.44%	Peroxiredoxin	Biosynthesis of secondary metabolites	osa00940
Phosphatidylinositol signaling system	1	4.00%	CDP-alcohol phosphatidyltransferase	Signal transduction	osa04070
Pyrimidine metabolism	1	1.22%	Glycoside transferase	Nucleotide metabolism	osa00240
Starch and sucrose metabolism	1	1.27%	1,4-alpha-D-glucan glucanohydrolase	Carbohydrate metabolism	osa00500
Ubiquitin mediated proteolysis	1	1.30%	Skp1 (Fragment).	Folding, sorting and degradation	osa04120
Valine, leucine and isoleucine biosynthesis	1	3.45%	Leucyl-tRNA synthetase, cytoplasmic	Amino acid metabolism	osa00290

TABLE IV: UP-REGULATED GENES INVOLVING IN MORE THAN TWO PATHWAYS

Gene Symbol and Short description	PathwayId	Pathway Name	Category
Os06g0492000 CDP-alcohol phosphatidyltransferase	osa00562	Inositol phosphate metabolism	Carbohydrate metabolism
	osa04070	Phosphatidylinositol signaling system	Signal transduction
Os02g0553200 Thylakoid-bound ascorbate peroxidase.	osa00053	Ascorbate and aldarate metabolism	Carbohydrate metabolism
	osa00480	Glutathione metabolism	Metabolism of other amino acids
Os01g0329000 Phospholipid/glycerol acyltransferase family	osa00561	Glycerolipid metabolism	Lipid metabolism
	osa00564	Glycerophospholipid metabolism	Lipid metabolism
Os07g0152900 Glycolate oxidase	osa00630	Glyoxylate and dicarboxylate metabolism	Carbohydrate metabolism
	osa04146	Peroxisome	
Os05g0241100 Leucyl-tRNA synthetase, cytoplasmic	osa00290	Valine, leucine and isoleucine biosynthesis	Amino acid metabolism
	osa00970	Aminoacyl-tRNA biosynthesis	Translation
Os07g0638400 Peroxiredoxin	osa00360	Phenylalanine metabolism	Amino acid metabolism
	osa00680	Methane metabolism	Energy metabolism
	osa00940	Phenylpropanoid biosynthesis	Biosynthesis of secondary metabolites
Os04g0630800 Anthocyanidin reductase.	osa00941	Flavonoid biosynthesis	Biosynthesis of secondary metabolites
	osa01061	Biosynthesis of phenylpropanoids	

TABLE V: PATHWAYS TO DOWN-REGULATED GENES (PARTIALLY, ONLY HITS PERCENT ≥5%)

Pathway Name	Hits	Percent	Description of the hit transcripts	Category	PathwayId
beta-Alanine metabolism	2	10.0%	Mitochondrial aldehyde dehydrogenase ALDH2a and Glutamate decarboxylase isozyme 3	Metabolism of Other Amino Acids	osa00410
Butanoate metabolism	2	6.9%	Glutamate decarboxylase isozyme 3 and Mitochondrial aldehyde dehydrogenase ALDH2a	Carbohydrate Metabolism	osa00650
Circadian rhythm - plant	1	6.67%	Response regulator receiver domain containing protein	Behavior	osa04712
Ether lipid metabolism	1	7.14%	Phospholipase D2	Lipid Metabolism	osa00565
Fatty acid biosynthesis	1	5.88%	Acyl-[acyl-carrier-protein] desaturase	Lipid Metabolism	osa00061
Fructose and mannose	2	4.88%	Mannose-6-phosphate isomerase and Hexokinase	Carbohydrate	osa00051

metabolism				Metabolism	
Histidine metabolism	1	6.25%	Mitochondrial aldehyde dehydrogenase ALDH2a	Amino Acid Metabolism	osa00340
Homologous recombination	2	8.33%	RecA bacterial DNA recombination protein family protein and 6-4 photolyase	Replication and Repair	osa03440
Limonene and pinene degradation	1	5.56%	Mitochondrial aldehyde dehydrogenase ALDH2a	Biosynthesis of Secondary Metabolites	osa00903
Lysine degradation	1	5.88%	Mitochondrial aldehyde dehydrogenase ALDH2a	Amino Acid Metabolism	osa00310
Nitrogen metabolism	2	6.9%	Phenylalanine ammonia-lyase 2 and Nitrate reductase 1	Energy Metabolism	osa00910
Non-homologous end-joining	1	12.5%	6-4 photolyase	Replication and Repair	osa03450
Pantothenate and CoA biosynthesis	1	5.88%	Aminotransferase, class IV family protein	Metabolism of Cofactors and Vitamins	osa00770
Phenylpropanoid biosynthesis	2	4.88%	4-coumarate--CoA ligase 2 and Phenylalanine ammonia-lyase 2	Biosynthesis of Secondary Metabolites	osa00940
Propanoate metabolism	1	5.88%	Mitochondrial aldehyde dehydrogenase ALDH2a	Carbohydrate Metabolism	osa00640
Steroid biosynthesis	1	6.67%	Sterol 4-alpha-methyl-oxidase	Lipid Metabolism	osa00100
Sulfur metabolism	1	5.56%	Serine acetyltransferase	Energy Metabolism	osa00920
Taurine and hypotaurine metabolism	1	14.29%	Glutamate decarboxylase isozyme 3	Metabolism of Other Amino Acids	osa00430
Thiamine metabolism	2	33.33%	Thiamine biosynthesis protein thiC and Thiamine pyrophosphokinase family protein	Metabolism of Cofactors and Vitamins	osa00730
Valine, leucine and isoleucine degradation	2	6.45%	Aminotransferase, class IV family protein and Mitochondrial aldehyde dehydrogenase ALDH2a	Amino Acid Metabolism	osa00280
Zeatin biosynthesis	1	14.29%	Cis-zeatin O-glucosyltransferase	Biosynthesis of Secondary Metabolites	osa00908

TABLE VI: DOWN-REGULATED GENES INVOLVING IN MORE THAN TWO PATHWAYS

Symbol	PathwayId	PathwayName	Category
Os01g0720700	osa00270	Cysteine and methionine metabolism	
	osa00920	Sulfur metabolism	Energy Metabolism
Os02g0697400	osa00130	Ubiquinone and other terpenoid-quinone biosynthesis	Metabolism of Cofactors and Vitamins
	osa00940	Phenylpropanoid biosynthesis	Biosynthesis of Secondary Metabolites
	osa01061	Biosynthesis of phenylpropanoids	
	osa01063	Biosynthesis of alkaloids derived from shikimate pathway	

Os11g0600900	osa00051	Fructose and mannose metabolism	Carbohydrate Metabolism
	osa00520	Amino sugar and nucleotide sugar metabolism	Carbohydrate Metabolism
Os02g0497500	osa03440	Homologous recombination (Japanese rice)	Replication and Repair
	osa03450	Non-homologous end-joining	Replication and Repair
Os07g0101500	osa00100	Steroid biosynthesis	Lipid Metabolism
	osa01066	Biosynthesis of alkaloids derived from terpenoid and polyketide	
Os04g0518400	osa00360	Phenylalanine metabolism	Amino Acid Metabolism
	osa00910	Nitrogen metabolism	Energy Metabolism
	osa00940	Phenylpropanoid biosynthesis	Biosynthesis of Secondary Metabolites
	osa01061	Biosynthesis of phenylpropanoids	
	osa01063	Biosynthesis of alkaloids derived from shikimate pathway	
	osa01064	Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid	
	osa01070	Biosynthesis of plant hormones	
Os04g0657100	osa00900	Terpenoid backbone biosynthesis	Biosynthesis of Secondary Metabolites
	osa01062	Biosynthesis of terpenoids and steroids	
	osa01066	Biosynthesis of alkaloids derived from terpenoid and polyketide	
	osa01070	Biosynthesis of plant hormones	
Os07g0446800	osa00010	Glycolysis / Gluconeogenesis	Carbohydrate Metabolism
	osa00051	Fructose and mannose metabolism	Carbohydrate Metabolism
	osa00052	Galactose metabolism	Carbohydrate Metabolism
	osa00500	Starch and sucrose metabolism	Carbohydrate Metabolism
	osa00520	Amino sugar and nucleotide sugar metabolism	Carbohydrate Metabolism
	osa01061	Biosynthesis of phenylpropanoids	
	osa01062	Biosynthesis of terpenoids and steroids	
	osa01063	Biosynthesis of alkaloids derived from shikimate pathway	
	osa01064	Biosynthesis of alkaloids derived from ornithine, lysine and nicotinic acid	
	osa01065	Biosynthesis of alkaloids derived from histidine and purine	
	osa01066	Biosynthesis of alkaloids derived from terpenoid and polyketide	
	osa01070	Biosynthesis of plant hormones	
Os05g0149400	osa00270	Cysteine and methionine metabolism	
	osa01070	Biosynthesis of plant hormones	
Os05g0244700	osa00280	Valine, leucine and isoleucine degradation	Amino Acid Metabolism
	osa00290	Valine, leucine and isoleucine biosynthesis	Amino Acid Metabolism
	osa00770	Pantothenate and CoA biosynthesis	Metabolism of Cofactors and Vitamins
	osa01063	Biosynthesis of alkaloids derived from shikimate pathway	
	osa01065	Biosynthesis of alkaloids derived from histidine and purine	
Os03g0236200	osa00250	Alanine, aspartate and glutamate metabolism	

	osa00410	beta-Alanine metabolism	Metabolism of Other Amino Acids
	osa00430	Taurine and hypotaurine metabolism	Metabolism of Other Amino Acids
	osa00650	Butanoate metabolism	Carbohydrate Metabolism
Os02g0730000	osa00010	Glycolysis / Gluconeogenesis	Carbohydrate Metabolism
	osa00053	Ascorbate and aldarate metabolism	Carbohydrate Metabolism
	osa00071	Fatty acid metabolism	Lipid Metabolism
	osa00280	Valine, leucine and isoleucine degradation	Amino Acid Metabolism
	osa00310	Lysine degradation	Amino Acid Metabolism
	osa00330	Arginine and proline metabolism	Amino Acid Metabolism
	osa00340	Histidine metabolism	Amino Acid Metabolism
	osa00380	Tryptophan metabolism	Amino Acid Metabolism
	osa00410	beta-Alanine metabolis	Metabolism of Other Amino Acids
	osa00561	Glycerolipid metabolism	Lipid Metabolism
	osa00620	Pyruvate metabolism	Carbohydrate Metabolism
	osa00640	Propanoate metabolism	Carbohydrate Metabolism
	osa00650	Butanoate metabolis	Carbohydrate Metabolism
	osa00903	Limonene and pinene degradation	Biosynthesis of Secondary Metabolites
Os01g0880800	osa00061	Fatty acid biosynthesis	Lipid Metabolism
	osa01040	Biosynthesis of unsaturated fatty acids	Lipid Metabolism
Os06g0604300	osa00564	Glycerophospholipid metabolism	Lipid Metabolism
	osa00565	Ether lipid metabolism	Lipid Metabolism
	osa04144	Endocytosis	

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