Time-course salt-treatment analysis provides insight into the salt tolerance of IPC at the transcriptional level

1. Analysis of gene expression and DEGs

Liu et al. conducted a transcriptome analysis of IPC under salt treatment in 2020 [1]. After completing the assembly and annotation of the IPC genome, We reanalyzed this set of data and continued to analyze the salt tolerance mechanism of IPC from the transcriptional level. In this analysis, two sets of material (Root and Leaf) were used for salt treatment and sampled at 5-time points (0h, 3h, 24h, 3d, 7d after salt treatment), with 3 biological replicates for each treatment. A total of 30 samples were used for this analysis. The samples at different treatment times were compared with the control samples (0h) to identify the DEGs (DEGs). In roots, the numbers of DEGs at 3h, 24h, 3d, and 7d after salt treatment were 2380, 1784, 2127, and 3616, respectively, among which the numbers of downregulated genes (DRGs) were 355, 553, 798 and 1592, and the numbers of up-regulated genes (URGs) were 2025, 1241, 1329 and 2024, respectively. In leaf tissue, the numbers of DEGs at 3h, 24h, 3d, and 7d after salt treatment were 220, 2360, 2085, and 3649, respectively, of which the numbers of down-regulated genes were 63, 1432, 1048, and 2105 respectively. The numbers of up-regulated genes were 157, 928, 1037, and 1544, respectively (Figure 1).

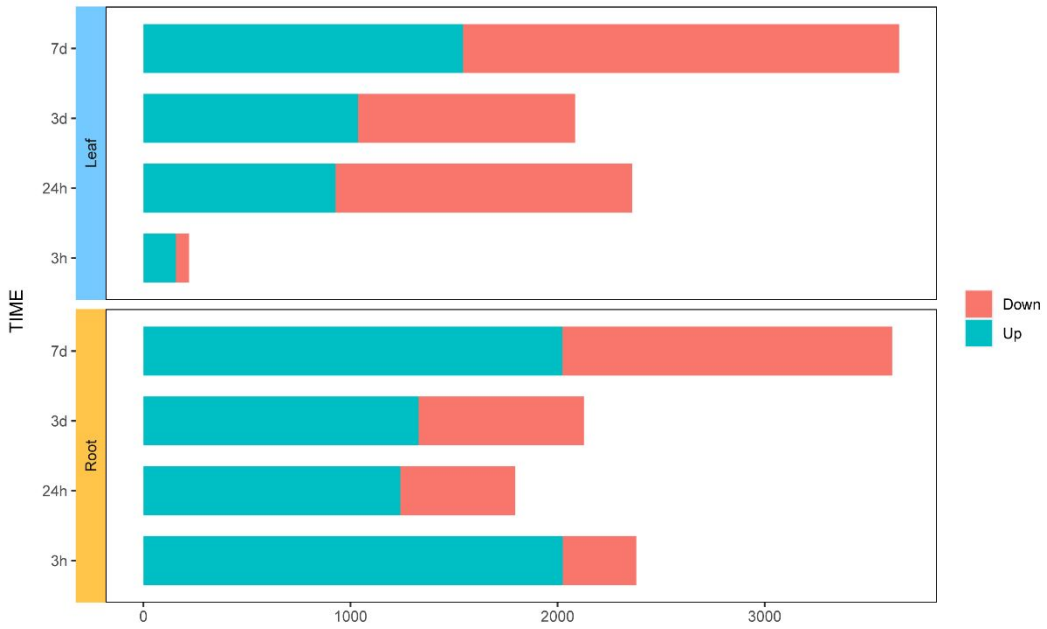

Figure 1*. The DEGs in the time-course treated roots and leaves of IPC*

Comparing the DEGs at different time points shows that the IPC roots and leaves respond to salt stress differently. There are only 220 DEGs in leaves at 3h, but the number of DEGs has reached 2360 at 7h, and then the number of DEGs in roots and leaves is similar. Figure 2 shows the percentage of shared DEGS of different sample groups. In roots, the proportion of shared DEGs with 3h of 24h, 3d, and 7d gradually increased and reached its maximum (945) at the 7d time point, accounting for 40% of the total DEGs at 7d. The shared differential genes in leaves are continuously increased. The number of DEGs at 3h is 100 and remains unchanged at 3d and 7d (Figure 2).

Figure 2*. Percentage of shared DEGs in different samples.*

2. Enrichment analysis of DEGs under salt stress

2.1 Time-course GO enrichment analysis of DEGs in roots

To understand the response mechanism of IPC to a high salt environment, the GOs of DEGs at each time point after salt treatment were enriched and analyzed. The GO enrichment analysis results of differential expression genes (DEGs), down-regulated expression genes (DRGs), and up-regulated expression genes (URGs) in IPC roots at different time points after salt treatment are shown in Figure 3.

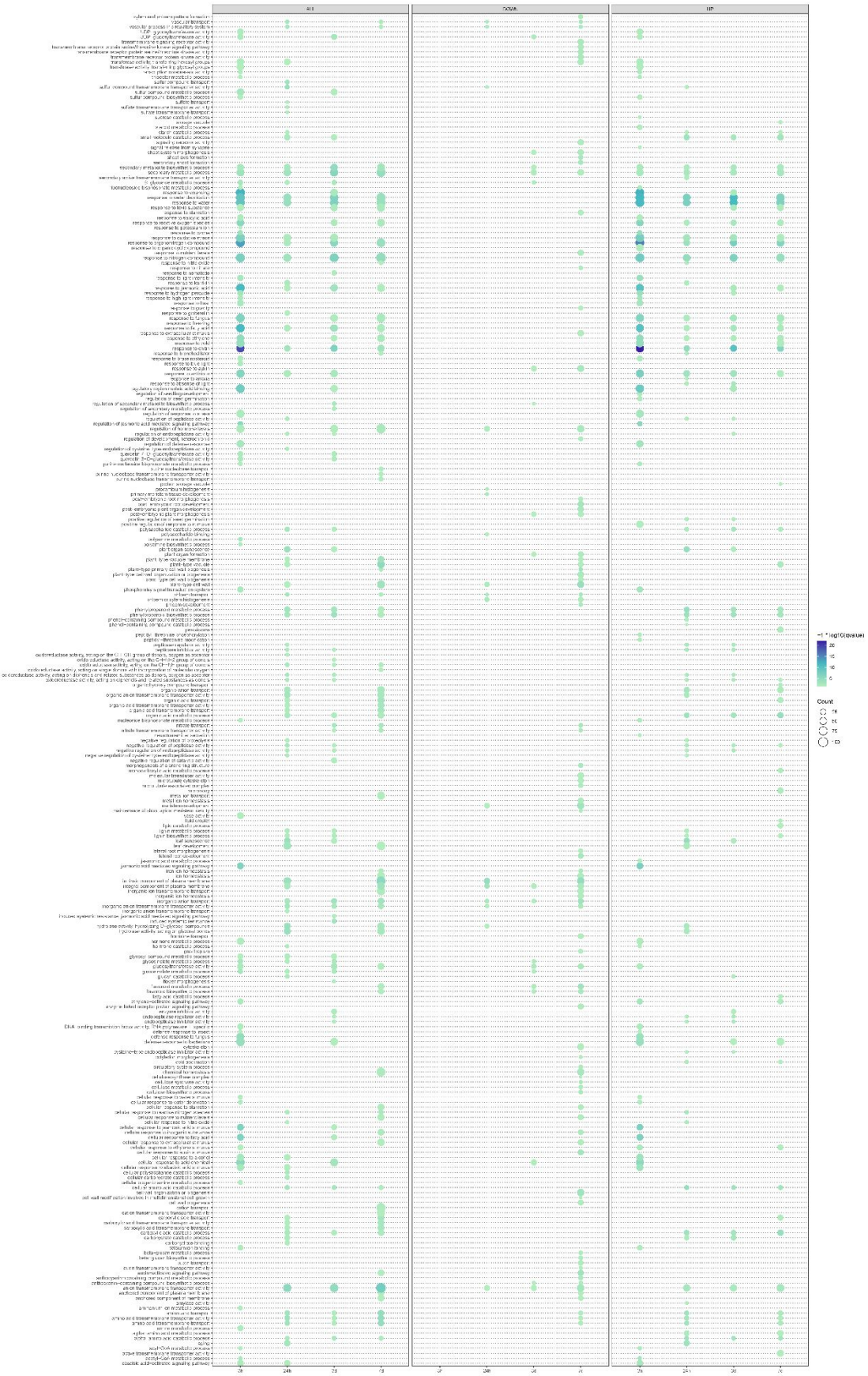

Figure 3. *The enriched GO terms of the DEGs in IPC roots under 3h, 24h, 3d, 7d treatment with 600mM NaCl.*

After 3 hours of salt treatment, the top 20 enriched GO terms with significant DEGs are response to chitin, response to organonitrogen compound, response to wounding, response to jasmonic acid, response to fatty acid, response to water deprivation, response to water, regulatory region nucleic acid binding, response to nitrogen compound, response to oxidative stress, cellular response to jasmonic acid stimulus, response to fungus, cellular response to fatty acid, jasmonic acid mediated signaling pathway, response to antibiotic, response to reactive oxygen species, cellular response to acid chemical, regulation of jasmonic acid mediated signaling pathway, response to ethylene, and defense response to fungus.

After 3 hours of salt treatment, the top 20 enriched GO terms with significant DRGs are anchored component of plasma membrane, plant-type cell wall, anchored component of membrane, cis-zeatin O-beta-D-glucosyltransferase activity, quercetin 3-O-glucosyltransferase activity, quercetin 7-O-glucosyltransferase activity, trans-zeatin O-beta-D-glucosyltransferase activity, calcium ion binding, glucuronosyltransferase activity, phosphoric diester hydrolase activity, and UDP-glucosyltransferase activity,

After 3 hours of salt treatment, the top 20 enriched GO terms with significant URGs are cellular response to abscisic acid stimulus, cellular response to alcohol, response to water deprivation, response to water, abscisic acid-activated signaling pathway, cellular response to acid chemical, positive regulation of signal transduction, positive regulation of response to stimulus, positive regulation of signaling, positive regulation of cell communication, plant-type cell wall organization, positive regulation of abscisic acid-activated signaling pathway, positive regulation of response to alcohol, positive regulation of cellular response to alcohol, cell wall organization, regulation of abscisic acid-activated signaling pathway, regulation of response to alcohol, and regulation of cellular response to alcohol.

After 24 hours of salt treatment, the top 20 enriched GO terms with significant DEGs are thylakoid, chloroplast thylakoid, plastid thylakoid, plastid thylakoid

membrane, photosynthetic membrane, chloroplast thylakoid membrane, thylakoid membrane, photosynthesis, light reaction, chloroplast envelope, photosynthesis, thylakoid lumen, photosynthesis, light harvesting, plastoglobule, chlorophyll-binding, photosynthesis, light harvesting in photosystem I, generation of precursor metabolites and energy, pigment binding, photosynthetic electron transport chain, photosynthetic electron transport in photosystem I, and NAD(P)H dehydrogenase complex (plastoquinone).

After 24 hours of salt treatment, the top 20 enriched GO terms with significant DRGs are thylakoid, chloroplast thylakoid, plastid thylakoid, photosynthetic membrane, plastid thylakoid membrane, chloroplast thylakoid membrane, thylakoid membrane, photosynthesis, light reaction, chloroplast envelope, photosynthesis, thylakoid lumen, plastoglobule, photosynthesis, light harvesting, generation of precursor metabolites and energy, photosynthesis, light harvesting in photosystem I, photosynthetic electron transport chain, pigment binding, chlorophyll-binding, photosynthetic electron transport in photosystem I, and protein domain specific binding.

After 24 hours of salt treatment, the top 20 enriched GO terms with significant URGs are response to water deprivation, response to water, glyoxysome, organic acid catabolic process, carboxylic acid catabolic process, small molecule catabolic process, cellular amino acid catabolic process, aromatic amino acid family catabolic process, response to mannitol, alpha-amino acid catabolic process, anion transmembrane transporter activity, tyrosine metabolic process, response to absence of light, proton transmembrane transporter activity, solute: proton symporter activity, monocarboxylic acid catabolic process, alpha-amino acid metabolic process, phenol-containing compound catabolic process, peptidyl-threonine phosphorylation, and peptidyl-threonine modification.

After 3 days of salt treatment, the top 20 enriched GO terms with significant DEGs are response to chitin, response to nitrogen compound, response to water deprivation, response to water, secondary metabolic process, response to

organonitrogen compound, anion transmembrane transporter activity, secondary metabolite biosynthetic process, response to fatty acid, phenylpropanoid biosynthetic process, inorganic anion transport, response to jasmonic acid, cellular response to acid chemical, induced systemic resistance, phenylpropanoid metabolic process, UDP-glucosyltransferase activity, glucosyltransferase activity, cellular amino acid catabolic process, alpha-amino acid catabolic process, and regulation of secondary metabolite biosynthetic process.

After 3 days of salt treatment, the top 20 enriched GO terms with significant DRGs are secondary metabolic process, flavonoid biosynthetic process, flavonoid metabolic process, cellular response to acid chemical, S-glycoside metabolic process, glycosinolate metabolic process, glucosinolate metabolic process, anthocyanin-containing compound biosynthetic process, inorganic anion transport, response to auxin, secondary metabolite biosynthetic process, plant organ formation, shoot system morphogenesis, regulation of secondary metabolite biosynthetic process, post-embryonic plant morphogenesis, integral component of plasma membrane, glucosyltransferase activity, UDP-glucosyltransferase activity, and anion transmembrane transporter activity.

After 3 days of salt treatment, the top 20 enriched GO terms with significant URGs are response to water deprivation, response to water, response to chitin, response to organonitrogen compound, response to nitrogen compound, phenylpropanoid biosynthetic process, cellular amino acid catabolic process, response to antibiotic, alpha-amino acid catabolic process, phenylpropanoid metabolic process, polysaccharide catabolic process, response to oxidative stress, organic acid catabolic process, carboxylic acid catabolic process, response to fungus, response to fatty acid, carbohydrate catabolic process, response to absence of light, small molecule catabolic process, and secondary metabolic process.

After 7 days of salt treatment, the top 20 enriched GO terms with significant DEGs are anion transmembrane transporter activity, intrinsic component of plasma membrane, plant-type vacuole, amino acid transmembrane transporter activity,

response to nitrogen compound, secondary metabolic process, amino acid transmembrane transport, amino acid transport, response to organonitrogen compound, response to water deprivation, response to water, integral component of plasma membrane, plant-type vacuole membrane, plant-type cell wall, response to chitin, carboxylic acid transport, inorganic anion transport, organic acid transport, secondary metabolite biosynthetic process, and nitrate transport.

After 7 days of salt treatment, the top 20 enriched GO terms with significant DRGs are intrinsic component of plasma membrane, plant-type cell wall, auxin-activated signaling pathway, inorganic anion transport, cell wall organization or biogenesis, flavonoid metabolic process, meristem development, plant-type primary cell wall biogenesis, post-embryonic root development, cellular response to auxin stimulus, anion transmembrane transporter activity, nitrate transport, secondary shoot formation, shoot axis formation, vascular transport, phloem transport, morphogenesis of a branching structure, post-embryonic plant organ development, lateral root development, and flavonoid biosynthetic process.

After 7 days of salt treatment, the top 20 enriched GO terms with significant URGs are response to water deprivation, response to water, response to organonitrogen compound, response to chitin, response to nitrogen compound, organic acid catabolic process, carboxylic acid catabolic process, cellular amino acid catabolic process, alpha-amino acid catabolic process, small molecule catabolic process, phenylpropanoid biosynthetic process, anion transmembrane transporter activity, secondary metabolic process, phenylpropanoid metabolic process, response to fungus, plant-type vacuole, secondary metabolite biosynthetic process, response to ethylene, amino acid transmembrane transport, and amino acid transport.

2.2 time-course GO enrichment analysis of leaves

The GO enrichment analysis results of differential expression genes (DEG), down-regulated expression genes (DRG), and up-regulated expression genes (URG) in IPC leaves at different time points after salt treatment are shown in Figure 4.

Figure 4. *The enriched GO terms of the DEGs in IPC leaves under 3h, 24h, 3d, 7d treatment with 600mM NaCl.*

After 3 hours of salt treatment, the top 20 enriched GO terms with significant DEGs are response to water deprivation, response to water, cellular response to abscisic acid stimulus, cellular response to alcohol, abscisic acid-activated signaling pathway, plant-type cell wall organization, cell wall organization, cellular response to acid chemical, external encapsulating structure organization, syncytium formation, positive regulation of signal transduction, phosphate ion transport, positive regulation of signaling, regulation of abscisic acid-activated signaling pathway, regulation of response to alcohol, regulation of cellular response to alcohol, positive regulation of cell communication, positive regulation of abscisic acid-activated signaling pathway, positive regulation of response to alcohol, and positive regulation of cellular response to alcohol.

After 3 hours of salt treatment, the top 20 enriched GO terms with significant DRGs are anchored component of plasma membrane, plant-type cell wall, anchored component of membrane, cis-zeatin O-beta-D-glucosyltransferase activity, quercetin 3-O-glucosyltransferase activity, quercetin 7-O-glucosyltransferase activity, trans-zeatin O-beta-D-glucosyltransferase activity, calcium ion binding, glucuronosyltransferase activity, phosphoric diester hydrolase activity, and UDP-glucosyltransferase activity.

After 3 hours of salt treatment, the top 20 enriched GO terms with significant URGs are cellular response to abscisic acid stimulus, cellular response to alcohol, response to water deprivation, response to water, abscisic acid-activated signaling pathway, cellular response to acid chemical, positive regulation of signal transduction, positive regulation of response to stimulus, positive regulation of signaling, positive regulation of cell communication, plant-type cell wall organization, positive regulation of abscisic acid-activated signaling pathway, positive regulation of response to alcohol, positive regulation of cellular response to alcohol, cell wall organization, regulation of abscisic acid-activated signaling pathway, regulation of response to alcohol, and regulation of cellular response to alcohol.

After 24 hours of salt treatment, the top 20 enriched GO terms with significant DEGs are thylakoid, chloroplast thylakoid, plastid thylakoid, plastid thylakoid membrane, photosynthetic membrane, chloroplast thylakoid membrane, thylakoid membrane, photosynthesis, light reaction, chloroplast envelope, photosynthesis, thylakoid lumen, photosynthesis, light harvesting, plastoglobule, chlorophyll binding, photosynthesis, light harvesting in photosystem I, generation of precursor metabolites and energy, pigment binding, photosynthetic electron transport chain, photosynthetic electron transport in photosystem I, and NAD(P)H dehydrogenase complex (plastoquinone).

After 24 hours of salt treatment, the top 20 enriched GO terms with significant DRGs are thylakoid, chloroplast thylakoid, plastid thylakoid, photosynthetic membrane, plastid thylakoid membrane, chloroplast thylakoid membrane, thylakoid membrane, photosynthesis, light reaction, chloroplast envelope, photosynthesis, thylakoid lumen, plastoglobule, photosynthesis, light harvesting, generation of precursor metabolites and energy, photosynthesis, light harvesting in photosystem I, photosynthetic electron transport chain, pigment binding, chlorophyll binding, photosynthetic electron transport in photosystem I, and protein domain specific binding.

After 24 hours of salt treatment, the top 20 enriched GO terms with significant URGs are response to water deprivation, response to water, glyoxysome, organic acid catabolic process, carboxylic acid catabolic process, small molecule catabolic process, cellular amino acid catabolic process, aromatic amino acid family catabolic process, response to mannitol, alpha-amino acid catabolic process, anion transmembrane transporter activity, tyrosine metabolic process, response to absence of light, proton transmembrane transporter activity, solute: proton symporter activity, monocarboxylic acid catabolic process, alpha-amino acid metabolic process, phenol-containing compound catabolic process, peptidyl-threonine phosphorylation, and peptidyl-threonine modification.

After 3 days of salt treatment, the top 20 enriched GO terms with significant DEGs are response to chitin, intrinsic component of plasma membrane, cutin biosynthetic process, anion transmembrane transporter activity, inorganic anion transmembrane transporter activity, integral component of plasma membrane, cellular response to extracellular stimulus, innate immune response, monocarboxylic acid metabolic process, suberin biosynthetic process, secondary metabolite biosynthetic process, cellular response to nutrient levels, cellular response to external stimulus, plant-type vacuole, defense response to bacterium, response to nitrogen compound, immune response, secondary active transmembrane transporter activity, inorganic anion transport, and secondary metabolic process.

After 3 days of salt treatment, the top 20 enriched GO terms with significant DRGs are defense response to bacterium, innate immune response, immune response, response to chitin, response to nitrogen compound, response to fungus, cellular response to phosphate starvation, response to organonitrogen compound, thylakoid, integral component of plasma membrane, intrinsic component of plasma membrane, cell death, cellular response to extracellular stimulus, cellular response to nutrient levels, cellular response to external stimulus, systemic acquired resistance, response to extracellular stimulus, response to nutrient levels, regulation of defense response, and NAD(P)H dehydrogenase complex (plastoquinone).

After 3 days of salt treatment, the top 20 enriched GO terms with significant URGs are cutin biosynthetic process, suberin biosynthetic process, response to water deprivation, response to water, active transmembrane transporter activity, fatty acid elongase activity, proton transmembrane transporter activity, anion transmembrane transporter activity, secondary active transmembrane transporter activity, solute:proton symporter activity, transferase activity, transferring acyl groups other than amino-acyl groups, transferase activity, transferring acyl groups, cellular amino acid catabolic process, inorganic anion

transmembrane transporter activity, active ion transmembrane transporter activity, solute:cation symporter activity, symporter activity, response to karrikin, monocarboxylic acid metabolic process, and nitrate transmembrane transporter activity.

After 7 days of salt treatment, the top 20 enriched GO terms with significant DEGs are thylakoid, plastid thylakoid membrane, photosynthetic membrane, plastid thylakoid, thylakoid membrane, chloroplast thylakoid membrane, chloroplast thylakoid, photosynthesis, light reaction, photosynthesis, photosynthetic electron transport chain, defense response to bacterium, anion transmembrane transporter activity, photosynthetic electron transport in photosystem I, photosynthesis, light harvesting, integral component of plasma membrane, apoplast, innate immune response, intrinsic component of plasma membrane, generation of precursor metabolites and energy, and response to water deprivation.

After 7 days of salt treatment, the top 20 enriched GO terms with significant DRGs are thylakoid, photosynthetic membrane, plastid thylakoid membrane, plastid thylakoid, thylakoid membrane, chloroplast thylakoid, chloroplast thylakoid membrane, photosynthesis, light reaction, photosynthesis, defense response to bacterium, generation of precursor metabolites and energy, photosynthetic electron transport chain, chloroplast envelope, photosynthetic electron transport in photosystem I, innate immune response, photosynthesis, light harvesting, regulation of defense response, thylakoid lumen, plastoglobule, and immune response.

After 7 days of salt treatment, the top 20 enriched GO terms with significant URGs are anion transmembrane transporter activity, response to water deprivation, response to water, monocarboxylic acid metabolic process, organic anion transport, suberin biosynthetic process, organic anion transmembrane transporter activity, oxidoreductase activity, acting on diphenols and related substances as donors, oxygen as acceptor, oxidoreductase activity, acting on

diphenols and related substances as donors, cutin biosynthetic process, fatty acid metabolic process, organic acid catabolic process, carboxylic acid catabolic process, small molecule catabolic process, cellular amino acid catabolic process, alpha-amino acid catabolic process, aromatic amino acid family catabolic process, amino acid transmembrane transporter activity, response to mannitol, and amino acid transmembrane transport.

2.3 time-course KEGG enrichment analysis of roots

The KEGG enrichment analysis results of differentially expressed genes (DEGs), down-regulated genes (DRGs), and up-regulated genes (URGs) in IPC roots at different time points after salt treatment are shown in Figure 5.

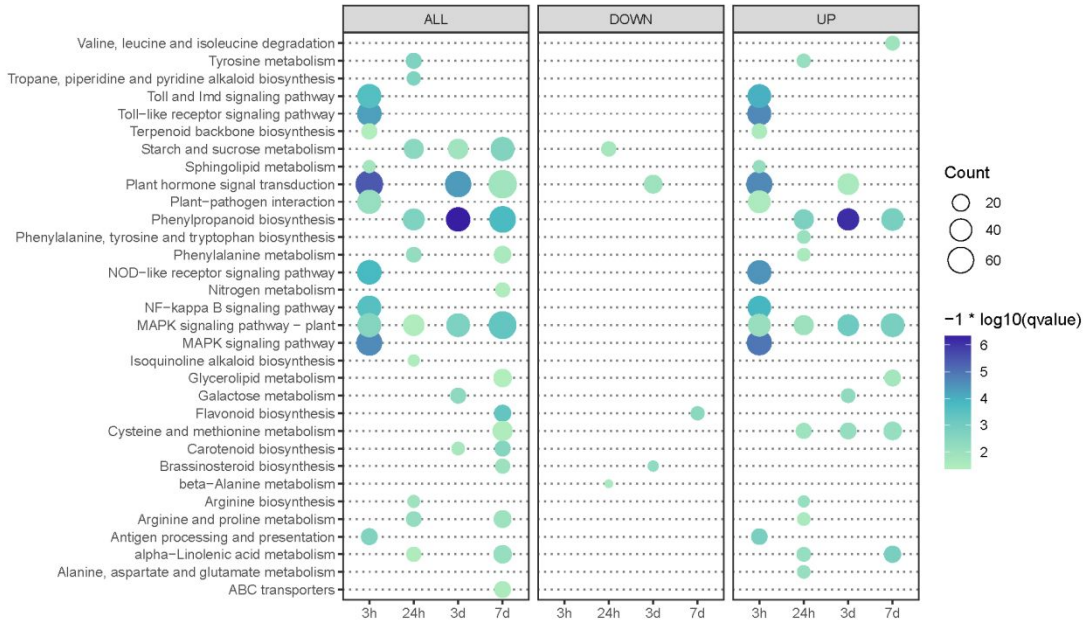

Figure 5. *The enriched KEGG terms of the DEGs in IPC roots under 3h, 24h, 3d, 7d treatment with 600mM NaCl.*

After 3 hours of salt treatment, the top 20 enriched KEGG terms with significant DEGs are Plant hormone signal transduction, MAPK signaling pathway, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, Toll and Imd signaling pathway, NF-kappa B signaling pathway, Antigen processing and

presentation, MAPK signaling pathway - plant, Plant-pathogen interaction, Sphingolipid metabolism, and Terpenoid backbone biosynthesis.

After 3 hours of salt treatment, No enriched KEGG terms with significant DRGs were found.

After 3 hours of salt treatment, the top 20 enriched KEGG terms with significant URGs are MAPK signaling pathway, Plant hormone signal transduction, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, Toll and Imd signaling pathway, NF-kappa B signaling pathway, Antigen processing and presentation, Sphingolipid metabolism, MAPK signaling pathway - plant, Plant-pathogen interaction, and Terpenoid backbone biosynthesis.

After 24 hours of salt treatment, the top 20 enriched KEGG terms with significant DEGs are Phenylpropanoid biosynthesis, Tyrosine metabolism, Tropane, piperidine and pyridine alkaloid biosynthesis, Starch and sucrose metabolism, Arginine and proline metabolism, Phenylalanine metabolism, Arginine biosynthesis, alpha-Linolenic acid metabolism, Isoquinoline alkaloid biosynthesis, and MAPK signaling pathway - plant.

After 24 hours of salt treatment, the enriched KEGG terms with significant DRGs are Starch and sucrose metabolism, and beta-Alanine metabolism.

After 24 hours of salt treatment, the top 20 enriched KEGG terms with significant URGs are Phenylpropanoid biosynthesis, Alanine, aspartate and glutamate metabolism, Arginine biosynthesis, alpha-Linolenic acid metabolism, Tyrosine metabolism, Phenylalanine, tyrosine and tryptophan biosynthesis, MAPK signaling pathway - plant, Cysteine and methionine metabolism, Arginine and proline metabolism, and Phenylalanine metabolism.

After 3 days of salt treatment, the top enriched KEGG terms with significant DEGs are Phenylpropanoid biosynthesis, Plant hormone signal transduction, MAPK signaling pathway - plant, Galactose metabolism, Starch and sucrose metabolism, and Carotenoid biosynthesis.

After 3 days of salt treatment, the top enriched KEGG terms with significant DRGs are Brassinosteroid biosynthesis, and Plant hormone signal transduction.

After 3 days of salt treatment, the top enriched KEGG terms with significant URGs are:Phenylpropanoid biosynthesis, MAPK signaling pathway - plant, Galactose metabolism, Cysteine and methionine metabolism, and Plant hormone signal transduction,

After 7 days of salt treatment, the top 20 enriched KEGG terms with significant DEGs are Phenylpropanoid biosynthesis, MAPK signaling pathway - plant, Flavonoid biosynthesis, Starch and sucrose metabolism, Carotenoid biosynthesis, alpha-Linolenic acid metabolism, Brassinosteroid biosynthesis, Arginine and proline metabolism, Plant hormone signal transduction, Phenylalanine metabolism, ABC transporters, Nitrogen metabolism, Cysteine and methionine metabolism, and Glycerolipid metabolism.

After 7 days of salt treatment, the top enriched KEGG terms with significant DRGs are: Flavonoid biosynthesis.

After 7 days of salt treatment, the top enriched KEGG terms with significant URGs are alpha-Linolenic acid metabolism, Phenylpropanoid biosynthesis, MAPK signaling pathway - plant, Cysteine and methionine metabolism, Valine, leucine and isoleucine degradation, and Glycerolipid metabolism.

2.4 time-course KEGG enrichment analysis of leaves

KEGG enrichment analysis results of differential expression genes (DEG), down-regulated expression genes (DRG), and up-regulated expression genes (URG) in IPC leaves at different time points after salt treatment are shown in Figure 6.

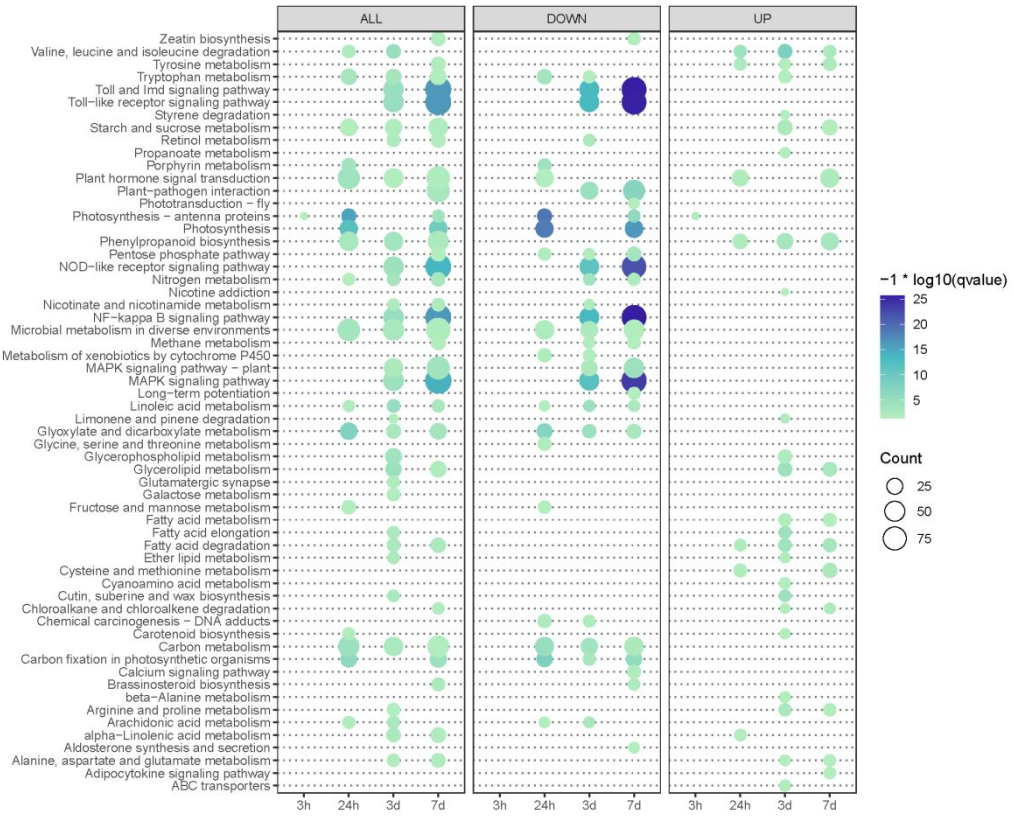

Figure 6. *The enriched KEGG terms of the DEGs in IPC leaves under 3h, 24h, 3d, 7d treatment with 600mM NaCl.*

After 3 hours of salt treatment, the top 20 enriched KEGG terms with significant DEGs are: Photosynthesis - antenna proteins.

After 3 hours of salt treatment, the top 20 enriched KEGG terms with significant DRGs are: NA

After 3 hours of salt treatment, the top 20 enriched KEGG terms with significant URGs are: Photosynthesis - antenna proteins.

After 24 hours of salt treatment, the top 20 enriched KEGG terms with significant DEGs are Photosynthesis - antenna proteins, Photosynthesis, Glyoxylate and dicarboxylate metabolism, Carbon fixation in photosynthetic organisms, Carbon metabolism, Plant hormone signal transduction, Porphyrin metabolism, Microbial metabolism in diverse environments, Tryptophan metabolism, Phenylpropanoid biosynthesis, Arachidonic acid metabolism, Linoleic acid metabolism, Valine,

leucine and isoleucine degradation, Carotenoid biosynthesis, Starch and sucrose metabolism, Nitrogen metabolism, and Fructose and mannose metabolism.

After 24 hours of salt treatment, the top 20 enriched KEGG terms with significant DRGs are Photosynthesis - antenna proteins, Photosynthesis, Carbon fixation in photosynthetic organisms, Glyoxylate and dicarboxylate metabolism, Carbon metabolism, Porphyrin metabolism, Tryptophan metabolism, Microbial metabolism in diverse environments, Chemical carcinogenesis - DNA adducts, Metabolism of xenobiotics by cytochrome P450, Pentose phosphate pathway, Glycine, serine and threonine metabolism, Linoleic acid metabolism, Plant hormone signal transduction, Arachidonic acid metabolism, and Fructose and mannose metabolism.

After 24 hours of salt treatment, the top 20 enriched KEGG terms with significant URGs are Valine, leucine and isoleucine degradation, Tyrosine metabolism, Fatty acid degradation, Cysteine and methionine metabolism, Plant hormone signal transduction, Phenylpropanoid biosynthesis, and alpha-Linolenic acid metabolism.

After 3 days of salt treatment, the top 20 enriched KEGG terms with significant DEGs are:Linoleic acid metabolism, NF-kappa B signaling pathway, Toll-like receptor signaling pathway, Toll and Imd signaling pathway, Valine, leucine and isoleucine degradation, Glycerolipid metabolism, NOD-like receptor signaling pathway, MAPK signaling pathway, Glycerophospholipid metabolism, Phenylpropanoid biosynthesis, Nitrogen metabolism, Tryptophan metabolism, Glyoxylate and dicarboxylate metabolism, Microbial metabolism in diverse environments, Arachidonic acid metabolism, Cutin, suberine and wax biosynthesis, Carbon metabolism, Fatty acid elongation, MAPK signaling pathway - plant, and alpha-Linolenic acid metabolism.

After 3 days of salt treatment, the top 20 enriched KEGG terms with significant DRGs are:NF-kappa B signaling pathway, Toll-like receptor signaling pathway,

Toll and Imd signaling pathway, MAPK signaling pathway, NOD-like receptor signaling pathway, Linoleic acid metabolism, Plant-pathogen interaction, Glyoxylate and dicarboxylate metabolism, Nitrogen metabolism, Carbon metabolism, Carbon fixation in photosynthetic organisms, Arachidonic acid metabolism, MAPK signaling pathway - plant, Retinol metabolism, Microbial metabolism in diverse environments, Nicotinate and nicotinamide metabolism, Chemical carcinogenesis - DNA adducts, Methane metabolism, Metabolism of xenobiotics by cytochrome P450, and Pentose phosphate pathway.

After 3 days of salt treatment, the top 20 enriched KEGG terms with significant URGs are:Valine, leucine and isoleucine degradation, Glycerolipid metabolism, Fatty acid elongation, Cutin, suberine and wax biosynthesis, Fatty acid degradation, Phenylpropanoid biosynthesis, Arginine and proline metabolism, Starch and sucrose metabolism, Limonene and pinene degradation, Glycerophospholipid metabolism, Fatty acid metabolism, beta-Alanine metabolism, Ether lipid metabolism, Chloroalkane and chloroalkene degradation, ABC transporters, Tryptophan metabolism, Carotenoid biosynthesis, Styrene degradation, Tyrosine metabolism, and Nicotine addiction.

After 7 days of salt treatment, the top 20 enriched KEGG terms with significant DEGs are:NF-kappa B signaling pathway, Toll and Imd signaling pathway, Toll-like receptor signaling pathway, MAPK signaling pathway, NOD-like receptor signaling pathway, Photosynthesis, Carbon fixation in photosynthetic organisms, photosynthesis - antenna proteins, MAPK signaling pathway - plant, Glyoxylate and dicarboxylate metabolism, Linoleic acid metabolism, Plant-pathogen interaction, Nitrogen metabolism, Brassinosteroid biosynthesis, Phenylpropanoid biosynthesis, Fatty acid degradation, Plant hormone signal transduction, Alanine, aspartate and glutamate metabolism, Microbial metabolism in diverse environments, and Glycerolipid metabolism.

After 7 days of salt treatment, the top 20 enriched KEGG terms with significant DRGs are: NF-kappa B signaling pathway, Toll and Imd signaling pathway, Toll-

like receptor signaling pathway, MAPK signaling pathway, NOD-like receptor signaling pathway, Photosynthesis, Plant-pathogen interaction, Photosynthesis – antenna proteins, Carbon fixation in photosynthetic organisms, MAPK signaling pathway – plant, Pentose phosphate pathway, Glyoxylate and dicarboxylate metabolism, Linoleic acid metabolism, Carbon metabolism, Nitrogen metabolism, Brassinosteroid biosynthesis, Calcium signaling pathway, Microbial metabolism in diverse environments, Phototransduction – fly, and Aldosterone synthesis and secretion.

After 7 days of salt treatment, the top 20 enriched KEGG terms with significant URGs are Fatty acid degradation, Phenylpropanoid biosynthesis, Glycerolipid metabolism, Valine, leucine and isoleucine degradation, Cysteine and methionine metabolism, Plant hormone signal transduction, Tyrosine metabolism, Chloroalkane and chloroalkene degradation, Arginine and proline metabolism, Starch and sucrose metabolism, Fatty acid metabolism, Adipocytokine signaling pathway, Alanine, and aspartate and glutamate metabolism.

3. Time-course expression analysis by Impulse DE2

3.1 GO enrichment analysis of roots

The temporal analysis of gene expression at different time points after salt treatment was conducted by the Impulse DE2 method. The DEGs in root tissues were classified into 1044 transition-up genes, 675 transition-down genes, 357 transient-up genes, and 36 transient-down genes (Figure 7). GO enrichment analysis was conducted for four groups of genes in roots.

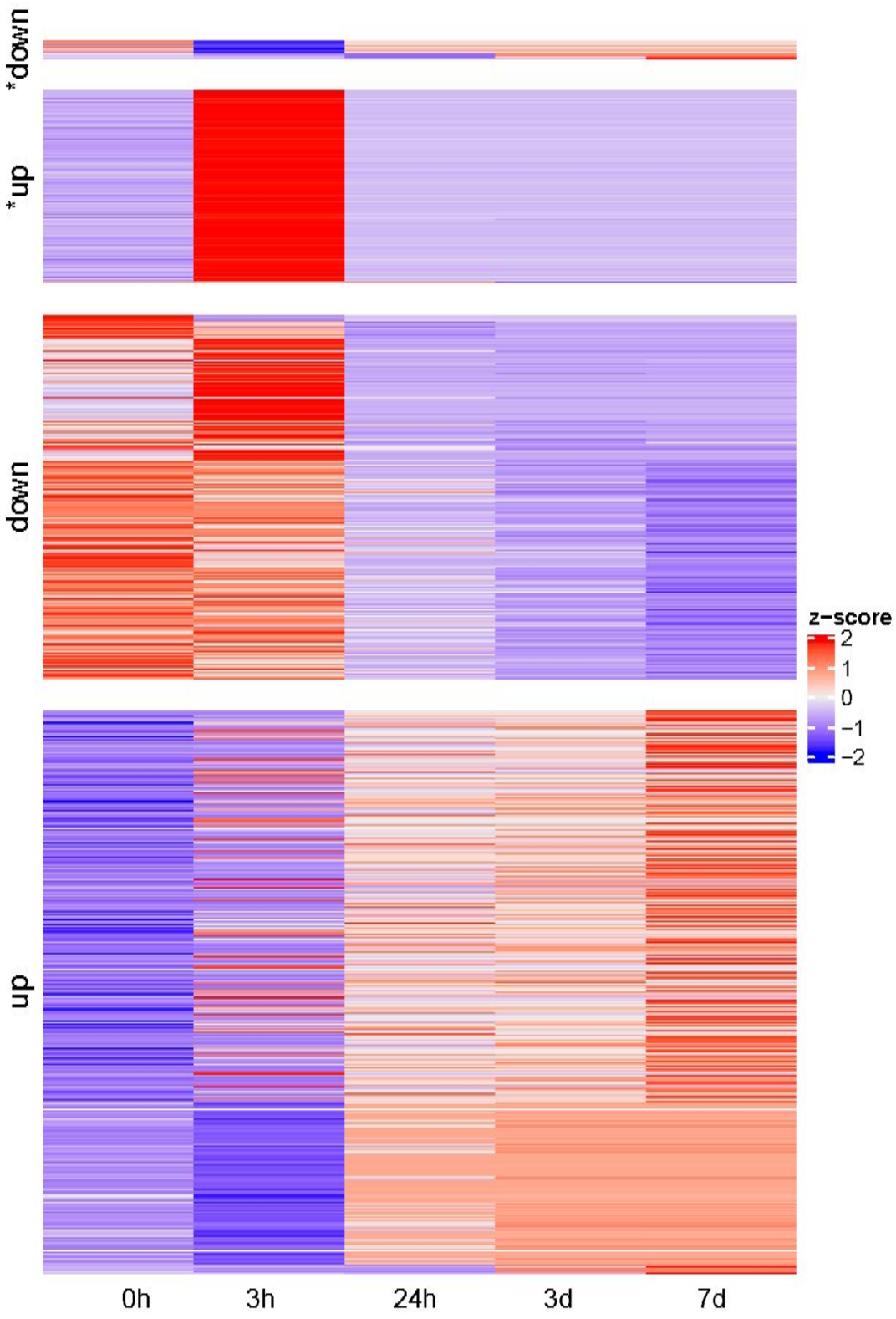

Figure 7. *The expression heat map of transient-down, transition-down, transient-up, and transition-up DEGs in IPC roots under salt treatment.*

None Gos were enriched with transient down genes in roots .

None Gos were enriched with transition down genes in roots are :

The top 10 enriched Gos with transient up genes in roots are response to water deprivation, response to water, response to wounding, response to chitin, response to organonitrogen compound, defense response to fungus, defense response to insect, response to cold, cellular response to jasmonic acid stimulus, response to fungus, response to jasmonic acid, cellular response to abscisic acid stimulus, cellular response to alcohol, response to high light intensity, jasmonic acid mediated signaling pathway, regulation of response to stress, response to oxidative stress, S-glycoside metabolic process, glycosinolate metabolic process, and glucosinolate metabolic process (Figure 8).

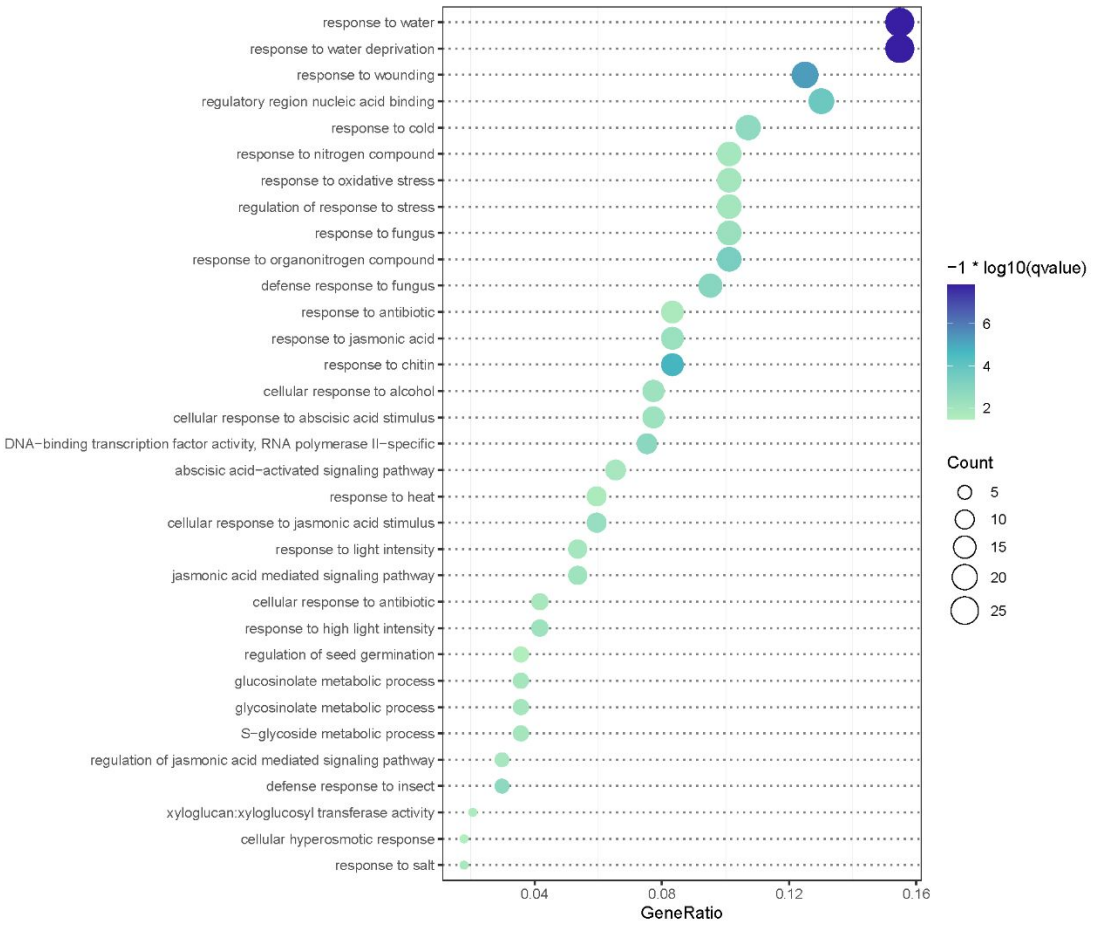

Figure 8. *The enriched GO terms of the transient-up DEGs in IPC root under salt treatment.*

The top 10 enriched Gos with f in roots are organic acid catabolic process, carboxylic acid catabolic process, small molecule catabolic process, cellular amino acid catabolic process, alpha-amino acid catabolic process, monocarboxylic acid catabolic process, response to water deprivation, response to water, fatty acid beta-oxidation, amino acid transmembrane transport, amino acid transport, fatty acid catabolic process, alpha-amino acid metabolic process, fatty acid oxidation, monocarboxylic acid metabolic process, polysaccharide catabolic process, cellular amino acid metabolic process, lipid oxidation, fatty acid metabolic process, and drug catabolic process (Figure 9).

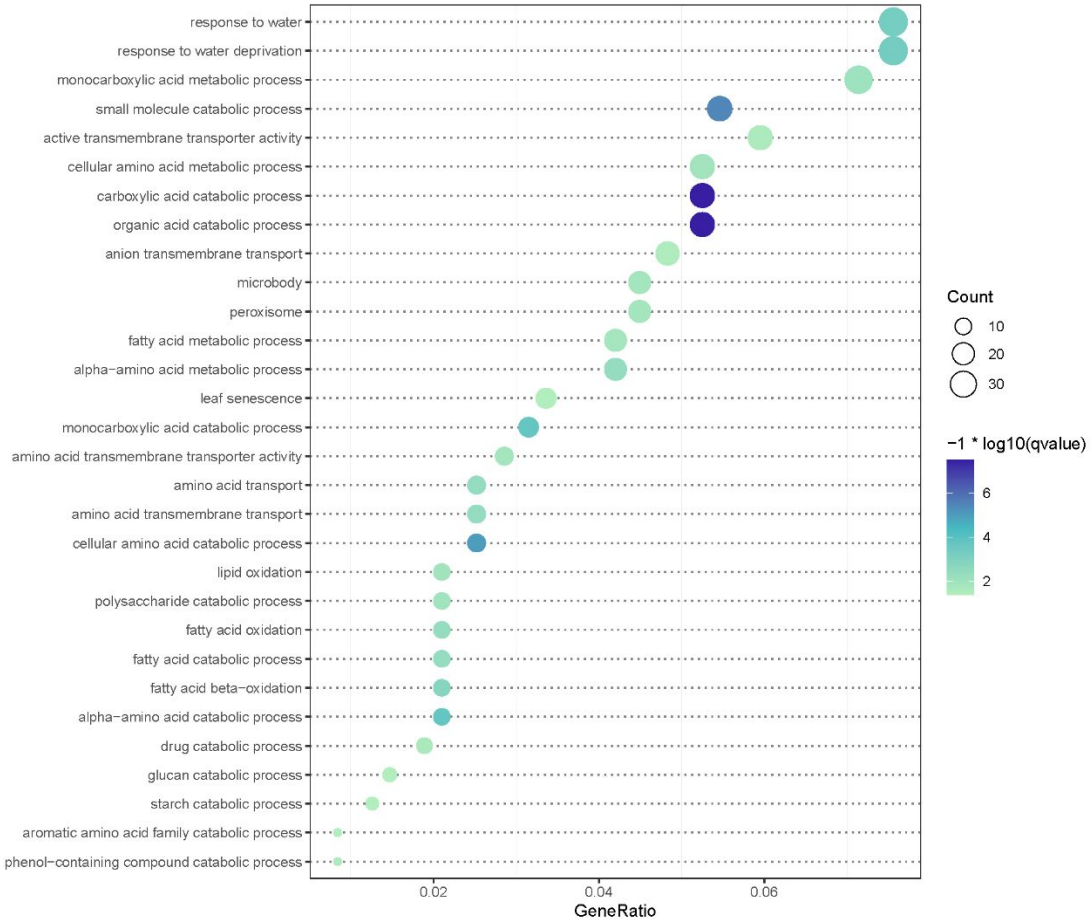

Figure 9. *The enriched GO terms of the transition-up DEGs in IPC root under salt treatment.*

3.2 KEGG enrichment analysis of roots

KEGG enrichment analysis was conducted for four groups of genes in roots.

No KEGGs were enriched with transient down genes in roots .

The top 10 enriched KEGGs with transition down genes in roots are (Figure 10): beta-Alanine metabolism.

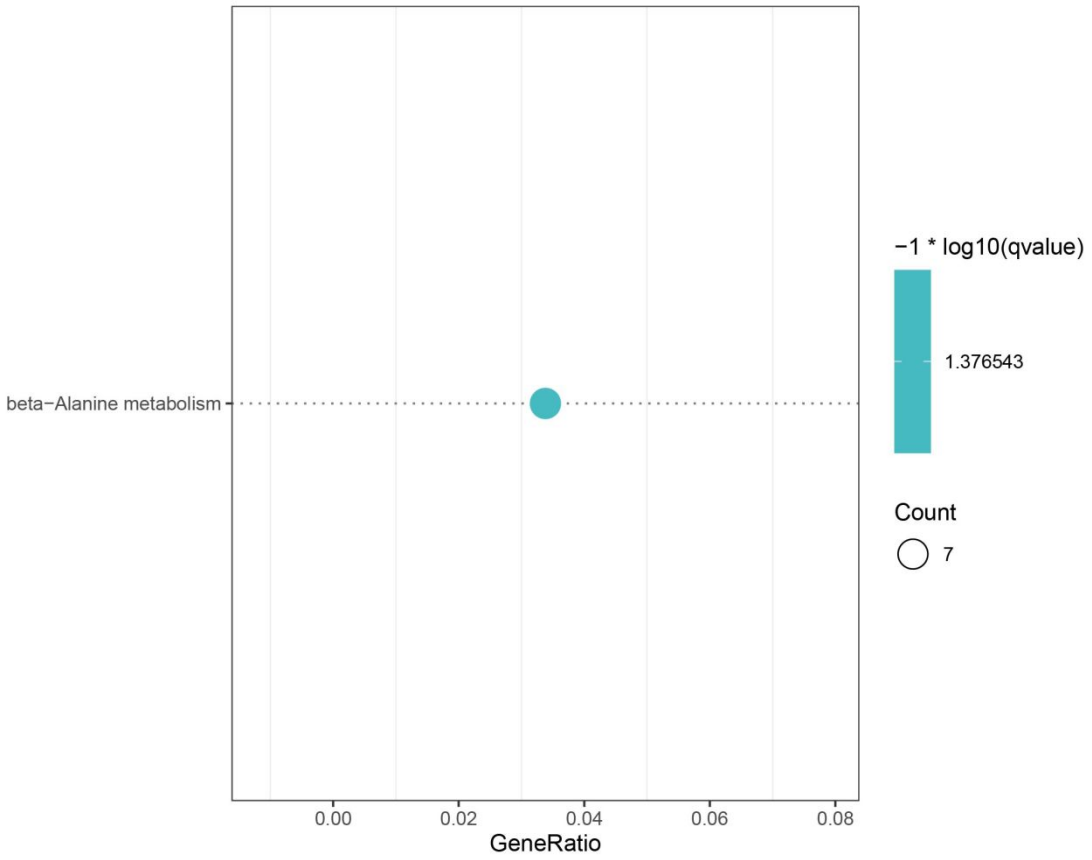

Figure 10*. The enriched KEGG terms of the transition-down DEGs in IPC roots under salt treatment.*

The enriched KEGGs with transient up genes in roots are (Figure 11): Plant hormone signal transduction.

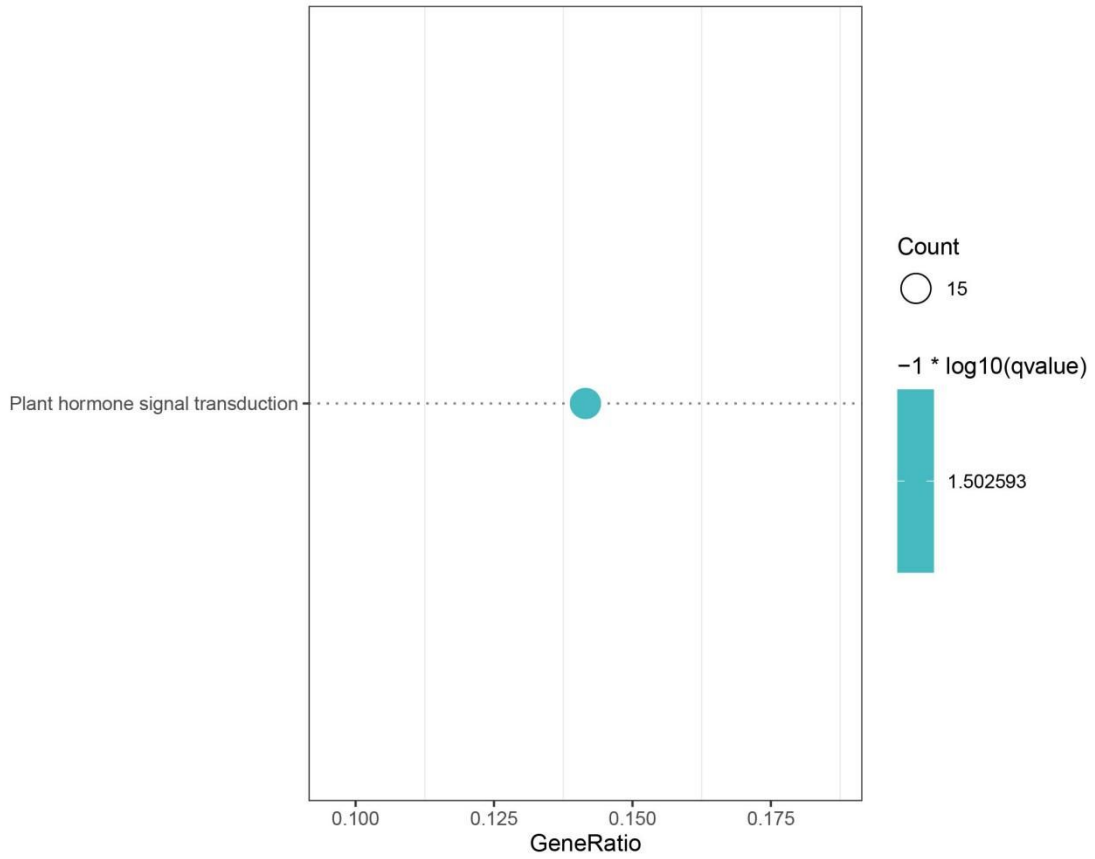

Figure 11. *The enriched KEGG terms of the transient-up DEGs in IPC roots under salt treatment.*

The enriched KEGGs with transition-up genes in roots are (Figure 12): Cysteine and methionine metabolism, and Nicotine addiction.

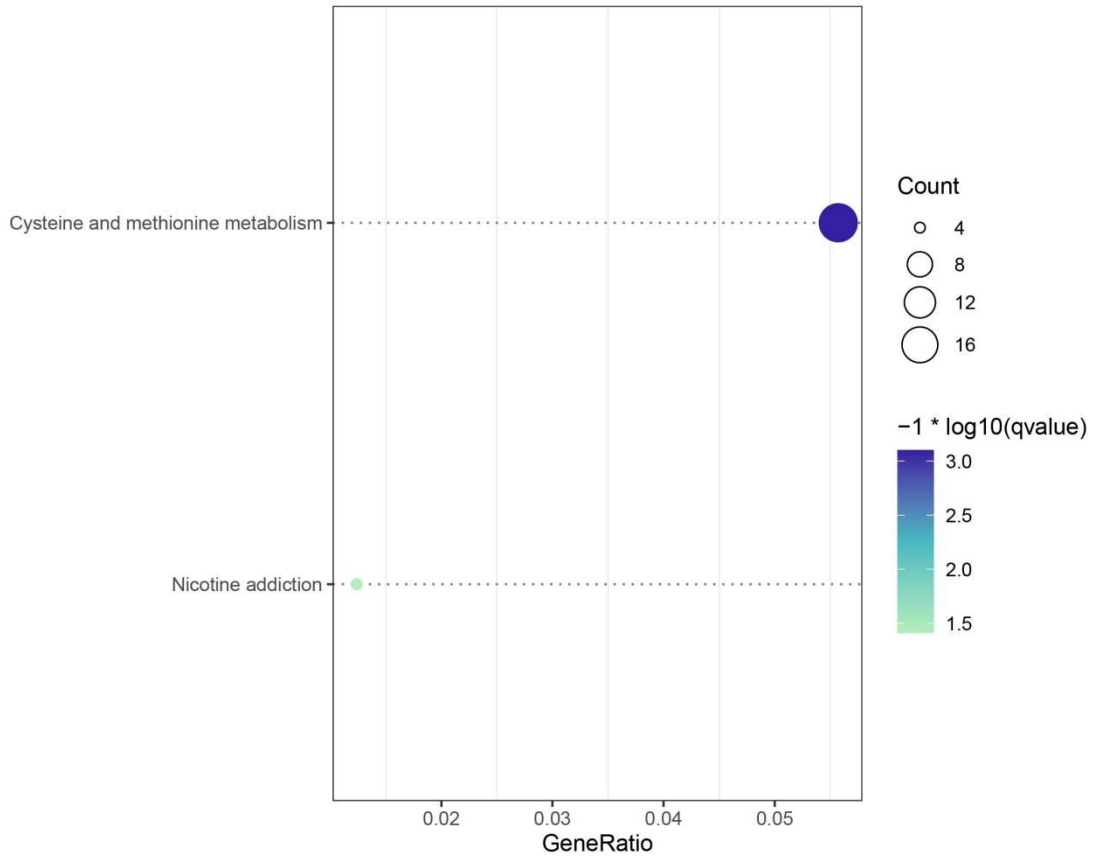

Figure 12. *The enriched KEGG terms of the transition-up DEGs in IPC roots under salt treatment.*

3.3 GO enrichment analysis of leaves

The temporal analysis of gene expression at different time points after salt treatment was conducted by the Impulse DE2 method. There were 997 transition-up genes, 1205 transition-down genes, 155 transient-up genes, and 17 transient-down genes in leaf tissues (Figure 13). GO enrichment analysis was conducted for four groups of genes in leaves.

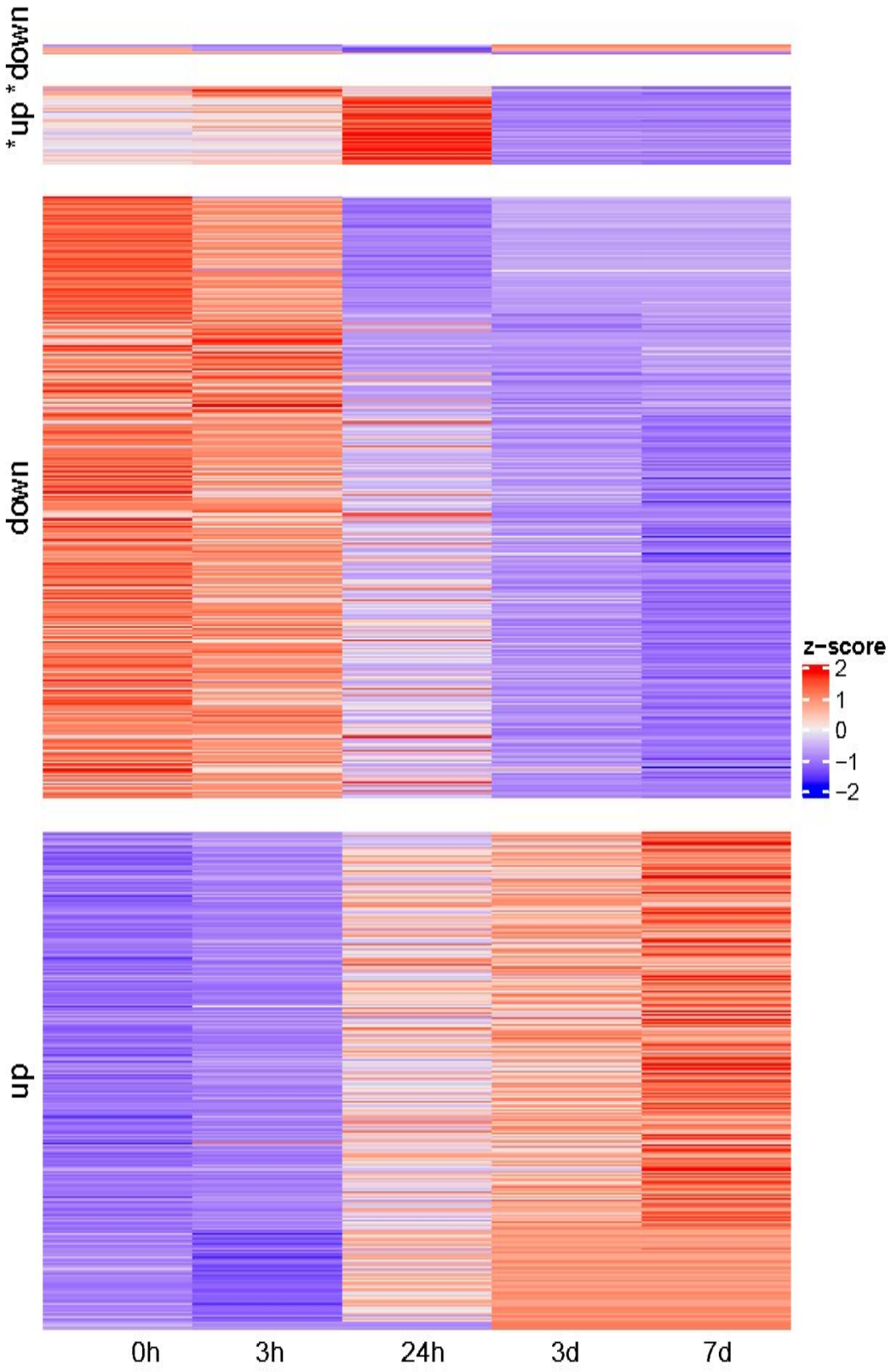

Figure 13. *The expression heat map of transient-down, transition-down, transient-up, and transition-up DEGs in IPC leaves under salt treatment.*

No Gos were enriched with transient down genes in leaves (Figure 14).

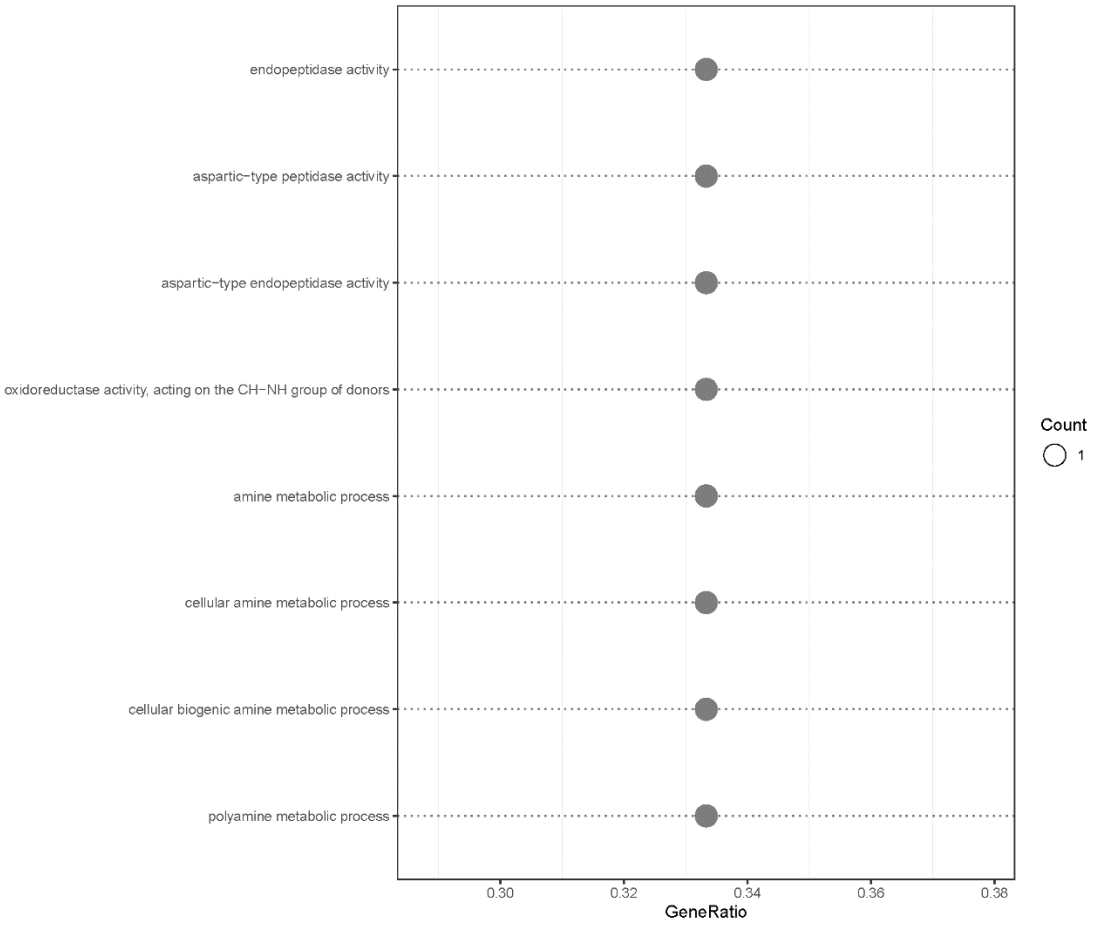

Figure 14. *The enriched GO terms of the transient-down DEGs in IPC leaves under salt treatment.*

The top 10 enriched Gos with transition down genes in leaves are thylakoid, plastid thylakoid, chloroplast thylakoid, photosynthetic membrane, thylakoid membrane, plastid thylakoid membrane, chloroplast thylakoid membrane, chloroplast envelope, immune response, defense response to bacterium, innate immune response, regulation of response to stress, generation of precursor metabolites and energy, photosynthesis, photosynthesis, light reaction, regulation of defense response, response to cold, response to water, response to water deprivation, response to wounding, and drug metabolic process (Figure 15).

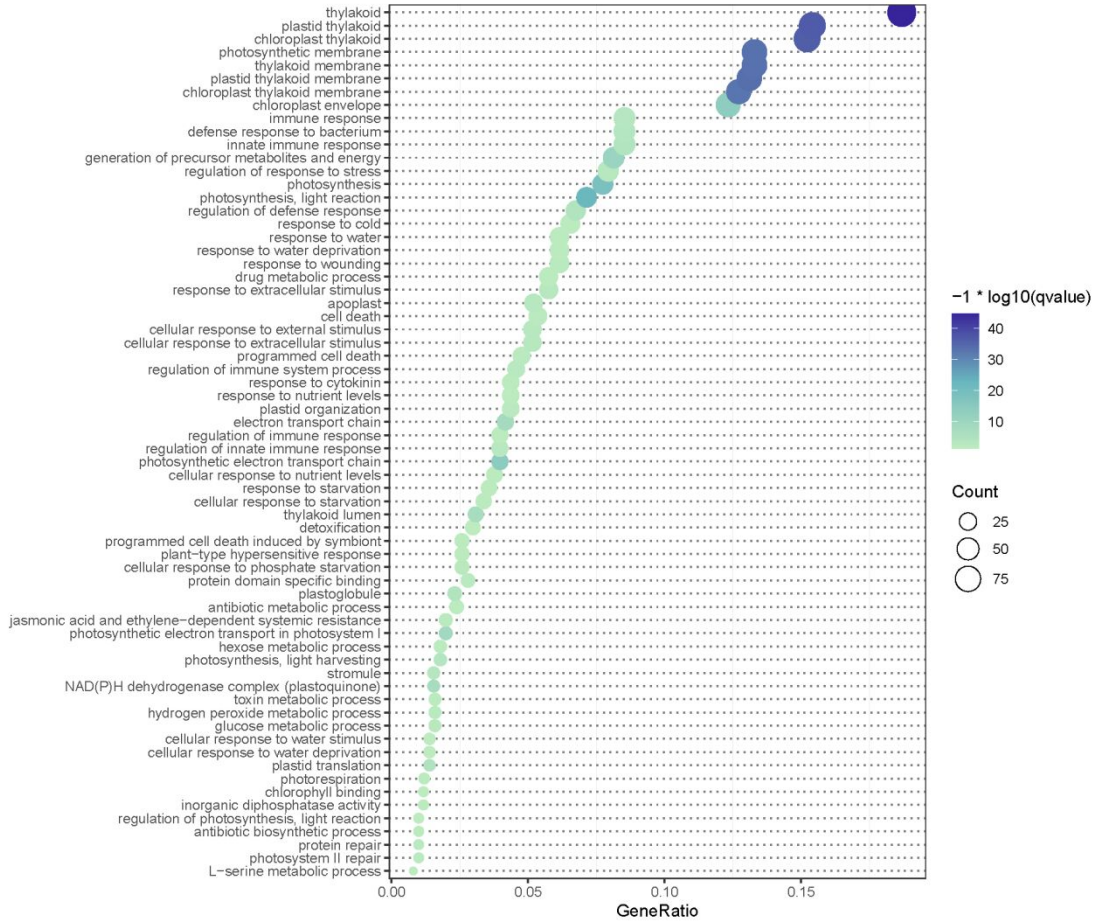

Figure 15*. The enriched GO terms of the transition-down DEGs in IPC leaves under salt treatment.*

The top 10 enriched Gos with transient up genes in leaves are (Figure 16):

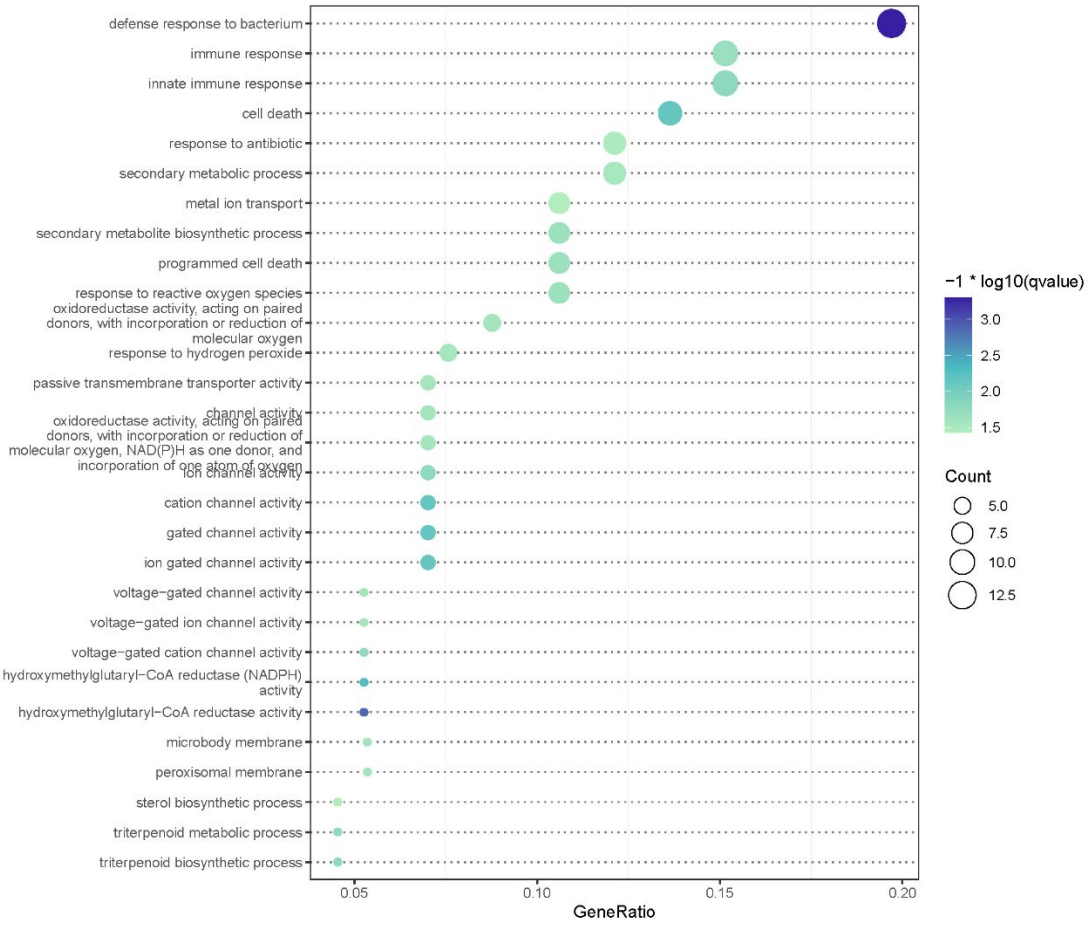

Figure 16. *The enriched GO terms of the transient-up DEGs in IPC leaves under salt treatment.*

The top 10 enriched Gos with transition-up genes in leaves are monocarboxylic acid metabolic process, response to water, response to water deprivation, cellular amino acid metabolic process, small molecule catabolic process, alpha−amino acid metabolic process, secondary metabolite biosynthetic process, carboxylic acid catabolic process, organic acid catabolic process, response to reactive oxygen species, proton transmembrane transport, monocarboxylic acid catabolic process, proton transmembrane transporter activity, cellular amino acid catabolic process, alpha−amino acid catabolic process, suberin biosynthetic process, fatty acid beta−oxidation, cellular biogenic amine biosynthetic process, amine biosynthetic process, and response to freezing (Figure 17).

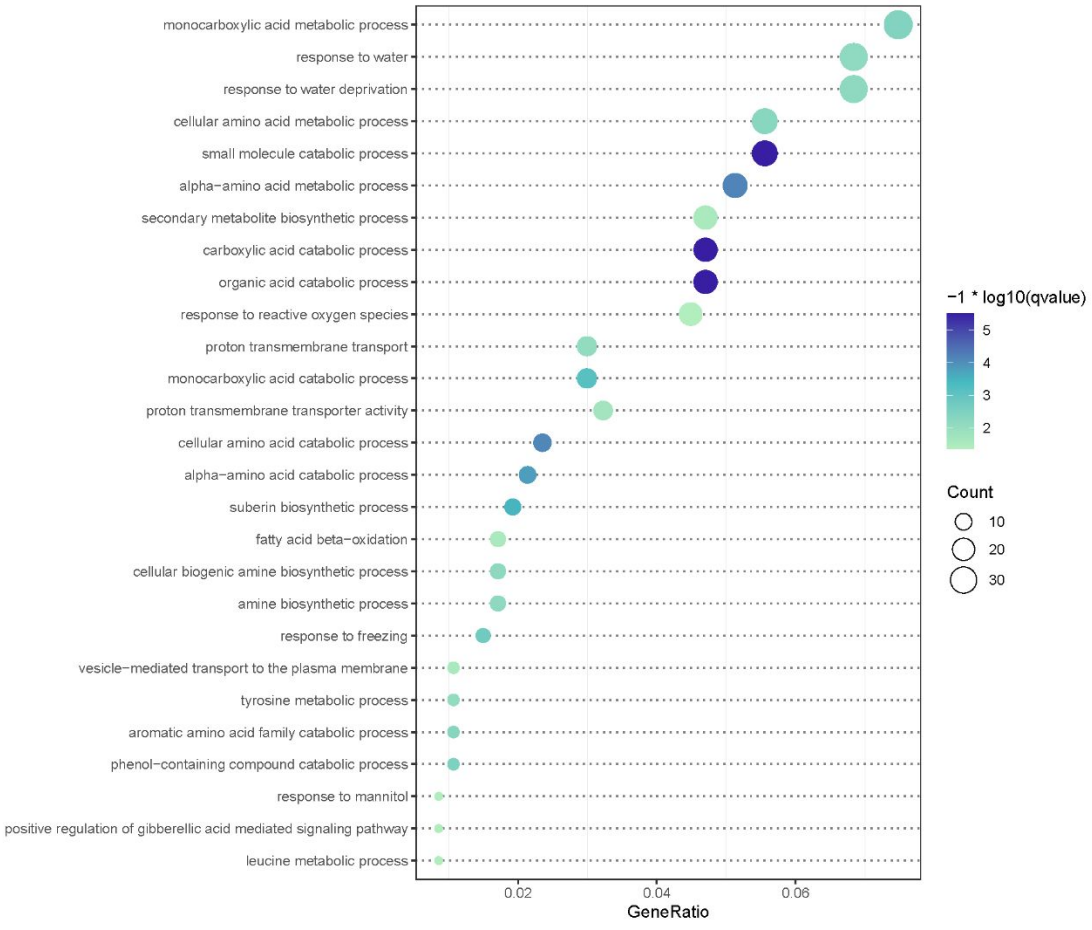

Figure 17. *The enriched GO terms of the transition-up DEGs in IPC leaves under salt treatment.*

3.4 KEGG enrichment analysis of leaves

KEGG enrichment analysis was conducted for four groups of genes in leaves. The top 10 enriched KEGGs with transient down genes in leaves are (Figure 18): beta-Alanine metabolism, and Arginine and proline metabolism.

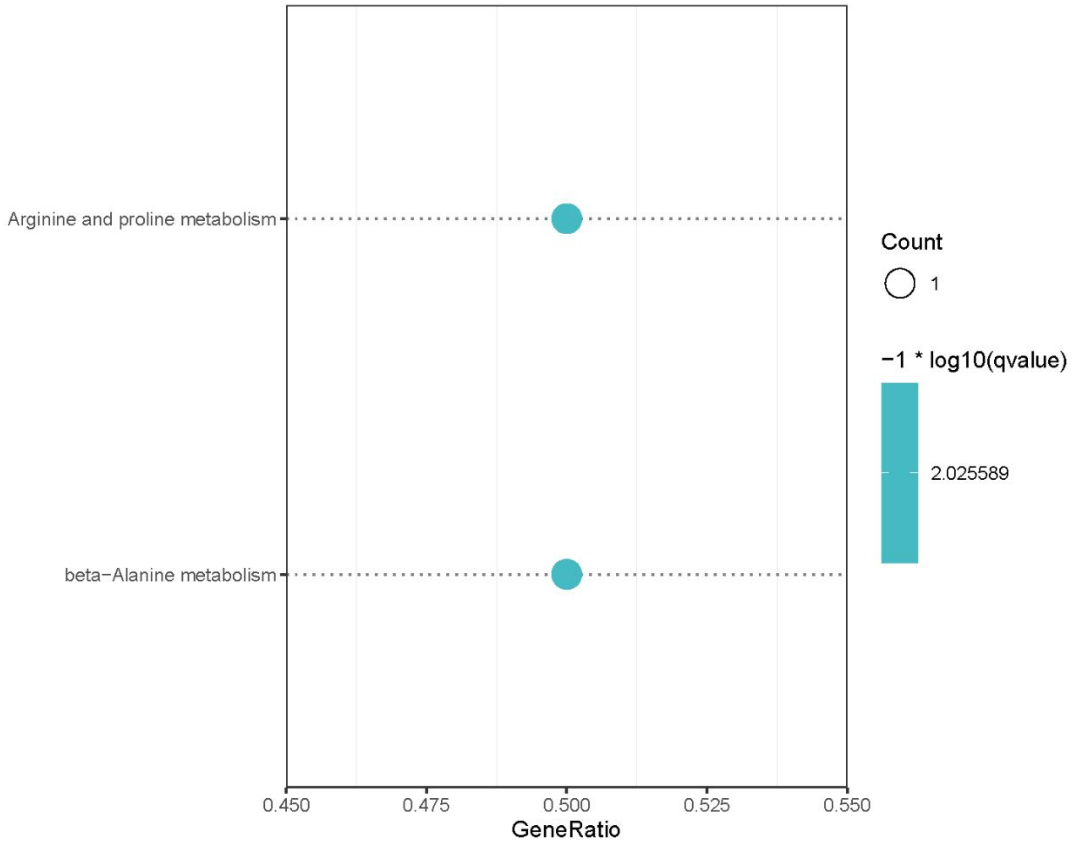

Figure 18. *The enriched KEGG terms of the transient-down DEGs in IPC leaves under salt treatment.*

The top enriched KEGGs with transition-down genes in leaves are shown in Figure 19.

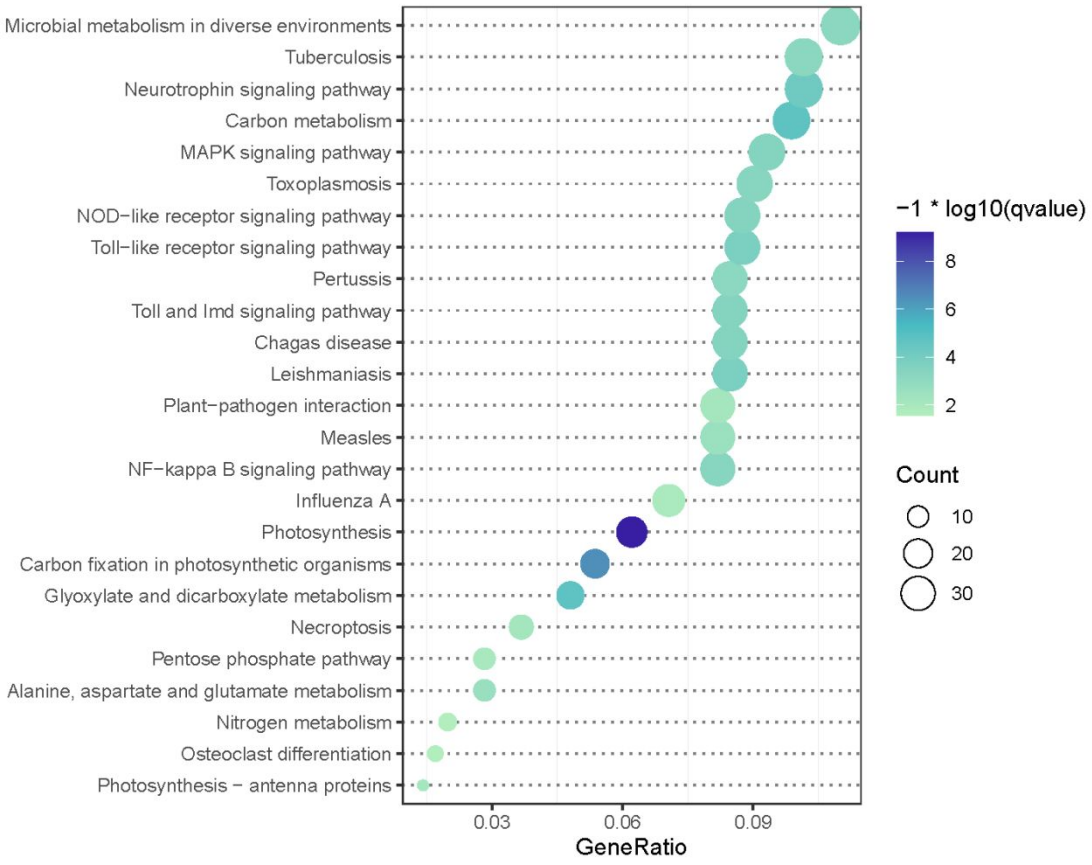

Figure 19. *The enriched KEGG terms of the transition-down DEGs in IPC leaves under salt treatment.*

The top 10 enriched KEGGs with transient up genes in leaves are (Figure 20): Tuberculosis, Chagas disease, Pertussis, Leishmaniasis, NF-kappa B signaling pathway, Neurotrophin signaling pathway, Toll-like receptor signaling pathway, Toll and Imd signaling pathway, Measles, Toxoplasmosis.

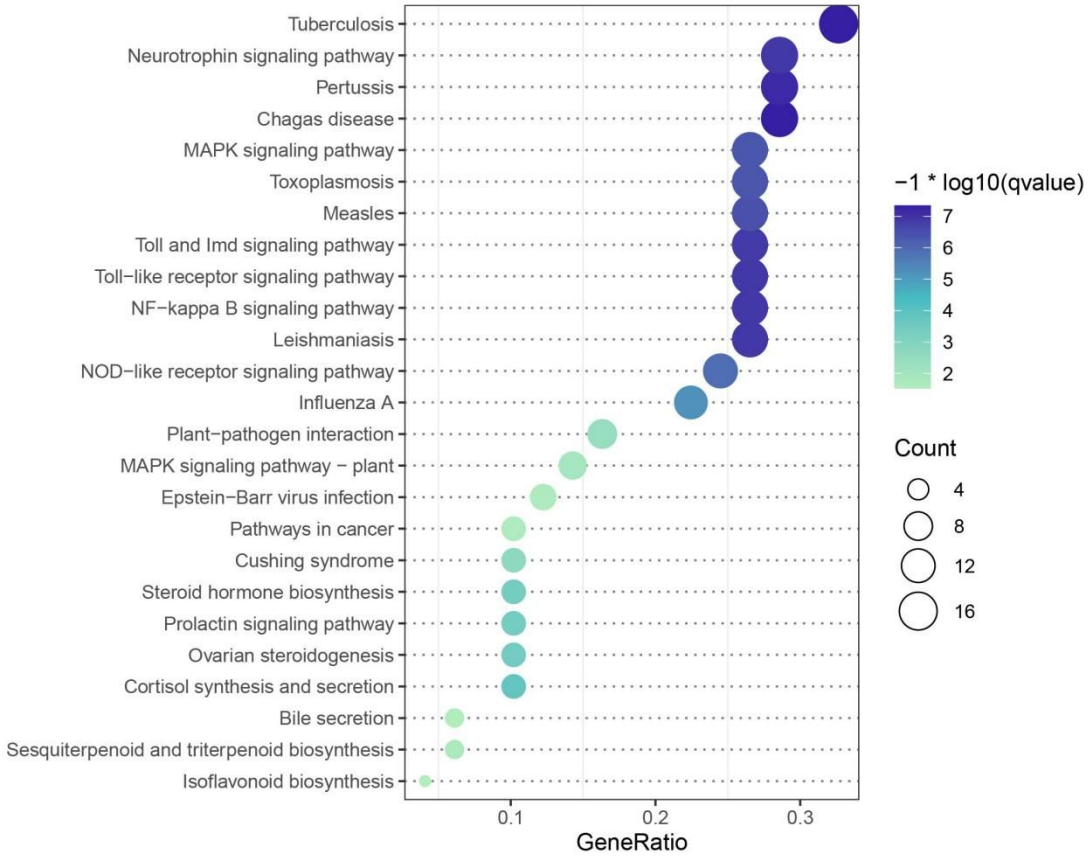

Figure 20. *The enriched KEGG terms of the transient-up DEGs in IPC leaves under salt treatment.*

The top 10 enriched KEGGs with transition-up genes in leaves are (Figure 21):Fatty acid degradation, Valine, leucine and isoleucine degradation, Tyrosine metabolism, mTOR signaling pathway, Arginine and proline metabolism, Chloroalkane and chloroalkene degradation, 2-Oxocarboxylic acid metabolism, Vibrio cholerae infection, Limonene and pinene degradation, beta-Alanine metabolism.

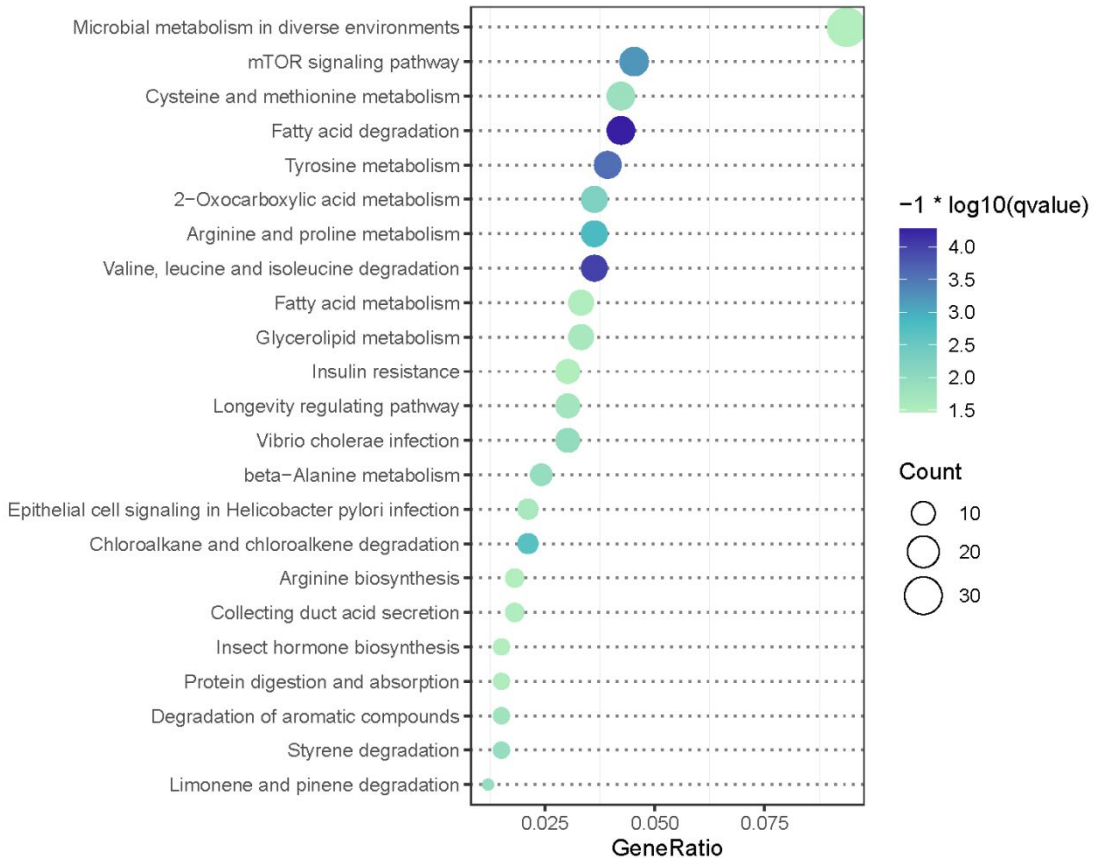

Figure 21. *The enriched KEGG terms of the transition-up DEGs in IPC leaves under salt treatment.*

4. Cluster analysis of gene expression time series under IPC

Time series analysis is to analyze the data of a group of time series samples. Generally, time series analysis can predict the trend of data through a large amount of data. However, time series analysis of biological data does not have such a strong prediction attribute due to data reasons, but we can see the expression patterns of different genes through sequence analysis. In this study, Mfuzz was used to analyze the time series of root and leaf salt processing data of IPC. According to the gene expression in roots, the DEGs were divided into four clusters. Cluster 1 and cluster 2 are the genes with expression that continuously increase or decrease. The number is 6319 and 8649, respectively. Cluster 3 represents the transient up-regulated genes, with the number of 6849. Cluster 4 are the transient down-regulated genes, with a total number of 6296 (Figure 22).

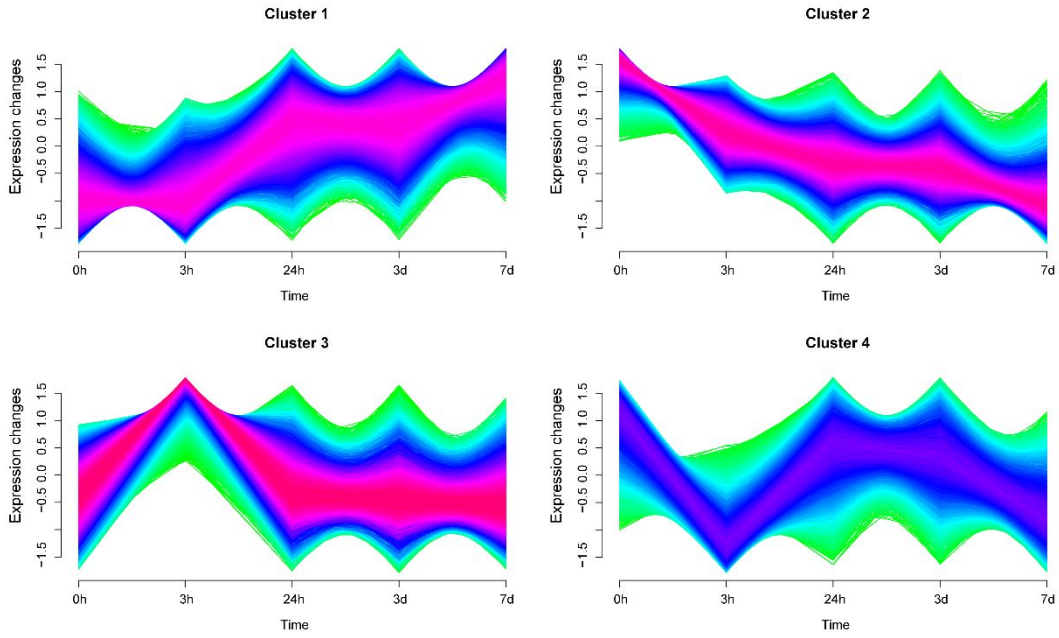

Figure 22. *Four gene clusters of DEGs in IPC roots under salt treatment.*

4.1 GO enrichment with roots

GO enrichment analysis was conducted with four groups of genes in roots (supplemental file 9).

The top 10 enriched Gos with Culster1 genes in roots are (Figure 23): cellular amino acid catabolic process, small molecule catabolic process, organic acid catabolic process, carboxylic acid catabolic process, secondary metabolite biosynthetic process, phenylpropanoid biosynthetic process, phenylpropanoid metabolic process, secondary metabolic process, alpha-amino acid catabolic process, leaf senescence.

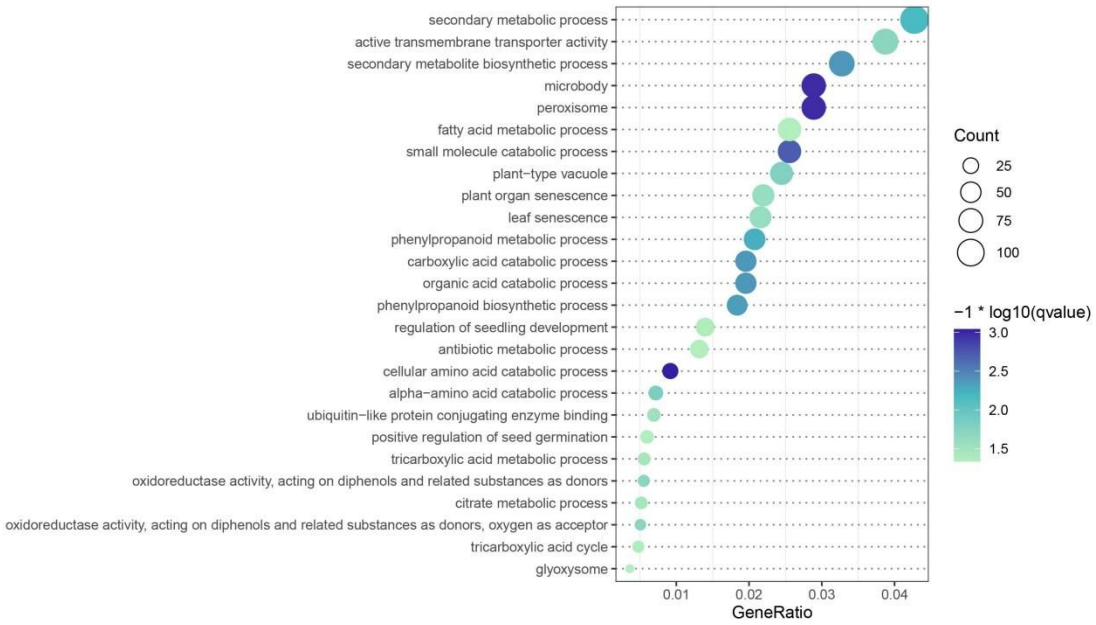

Figure 23. *The enriched GO terms of the Cluster 1 DEGs in IPC roots under salt treatment.*

The top 10 enriched Gos with Culster2 genes in roots are (Figure 24): cell cycle process, mitotic cell cycle process, microtubule-based movement, mitotic cell cycle, cytoskeleton-dependent cytokinesis, microtubule-based process, mitotic cytokinesis, cytokinesis, supramolecular fiber organization, cytoskeleton organization.

Figure 24. *The enriched GO terms of the Cluster 2 DEGs in IPC roots under salt treatment.*

The top 10 enriched Gos with Culster3 genes in roots are (Figure 25): response to chitin, response to organonitrogen compound, response to high light intensity, response to water, nucleotide-sugar biosynthetic process, response to water deprivation, response to brassinosteroid, response to wounding, response to ethylene, nucleotide-sugar metabolic process.

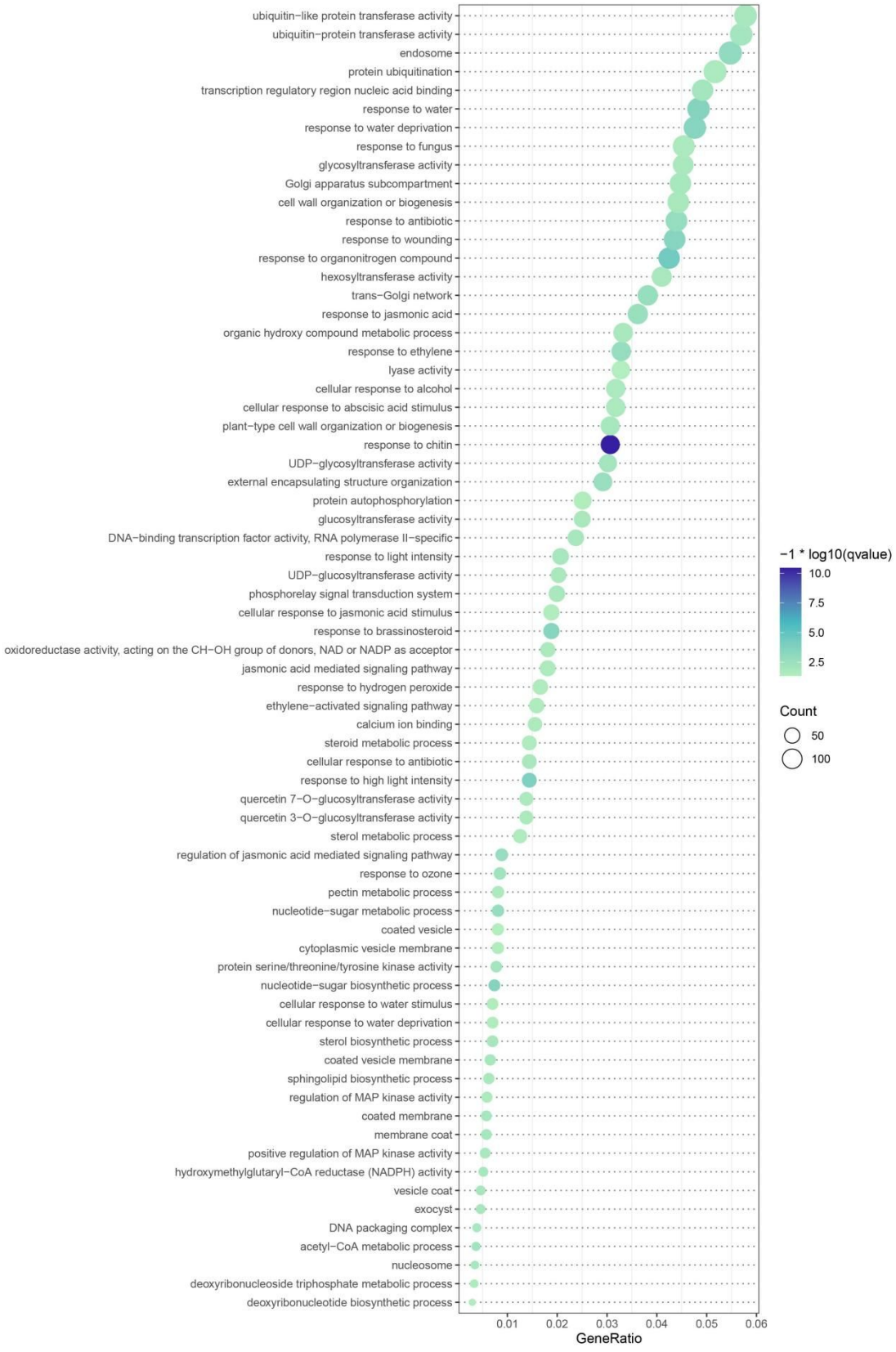

Figure 25. *The enriched GO terms of the Cluster 3 DEGs in IPC roots under salt treatment.*

The top 10 enriched Gos with Culster4 genes in roots are (Figure 26): ribonucleoprotein complex biogenesis, ribosome biogenesis, ncRNA metabolic process, translation, mRNA metabolic process, peptide biosynthetic process, amide biosynthetic process, ncRNA processing, mRNA processing, peptide metabolic process.

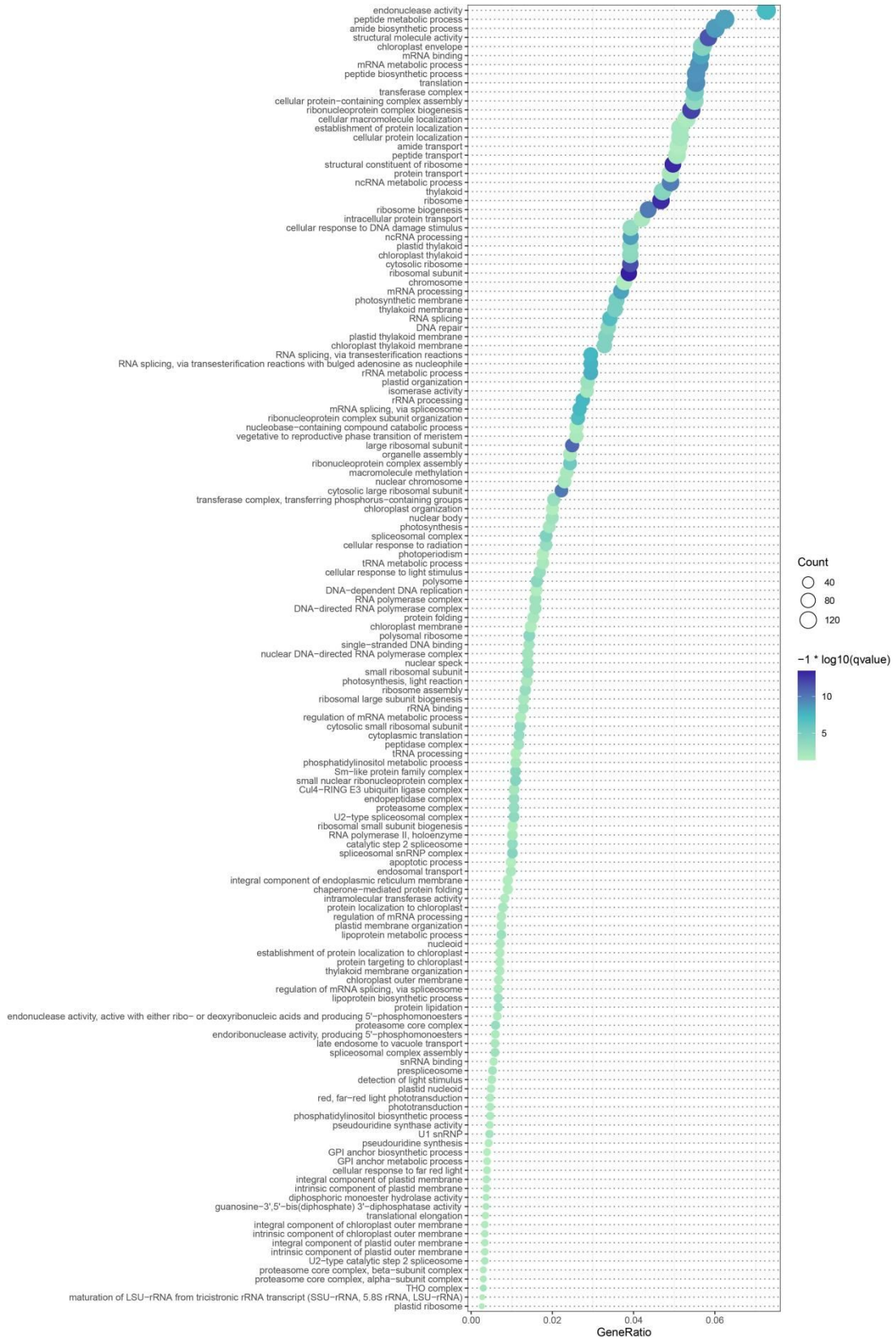

Figure 26. *The enriched GO terms of the Cluster 4 DEGs in IPC roots under salt treatment.*

4.2 KEGG enrichment with roots

KEGG enrichment analysis was conducted with four groups of genes in roots.

The enriched KEGGs with Culster 1 genes in roots are (Figure 27): Phenylpropanoid biosynthesis.

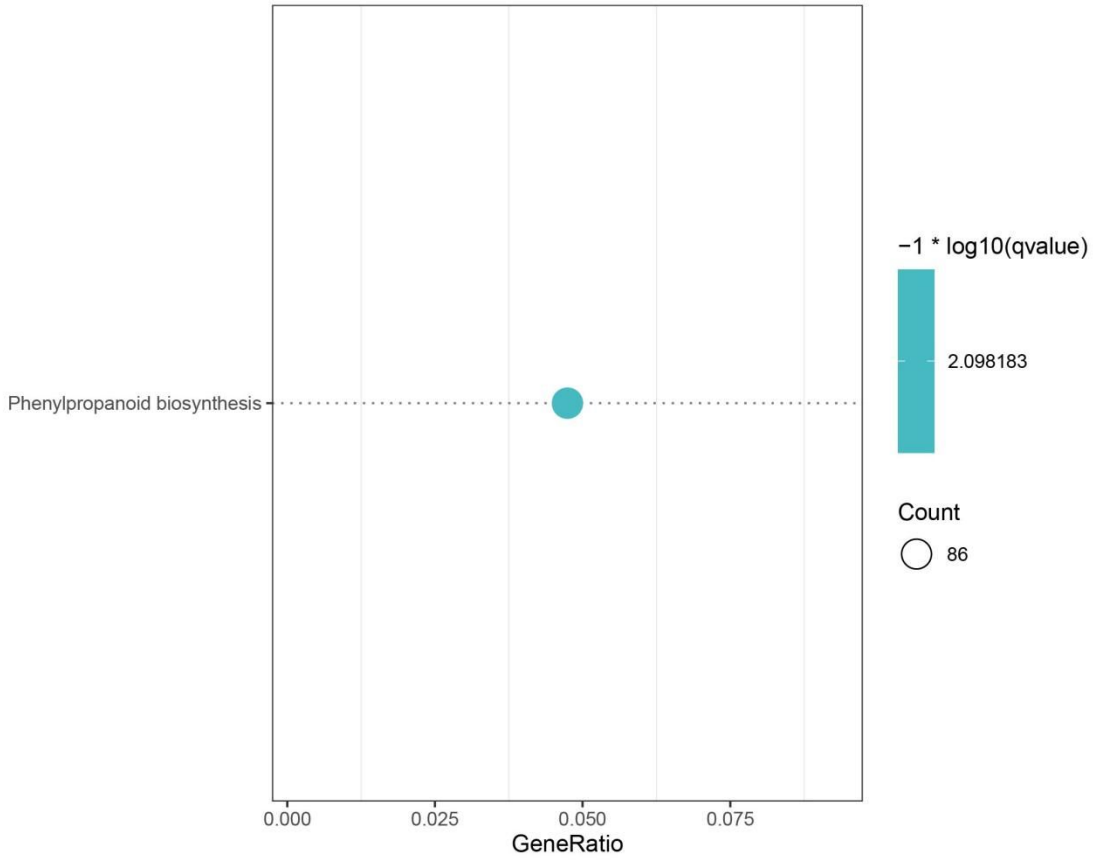

Figure 27. *The enriched KEGG terms of the Cluster 1 DEGs in IPC roots under salt treatment.*

The top 10 enriched KEGGs with Culster2 genes in roots are (Figure 28): Progesterone-mediated oocyte maturation, Ribosome biogenesis in eukaryotes.

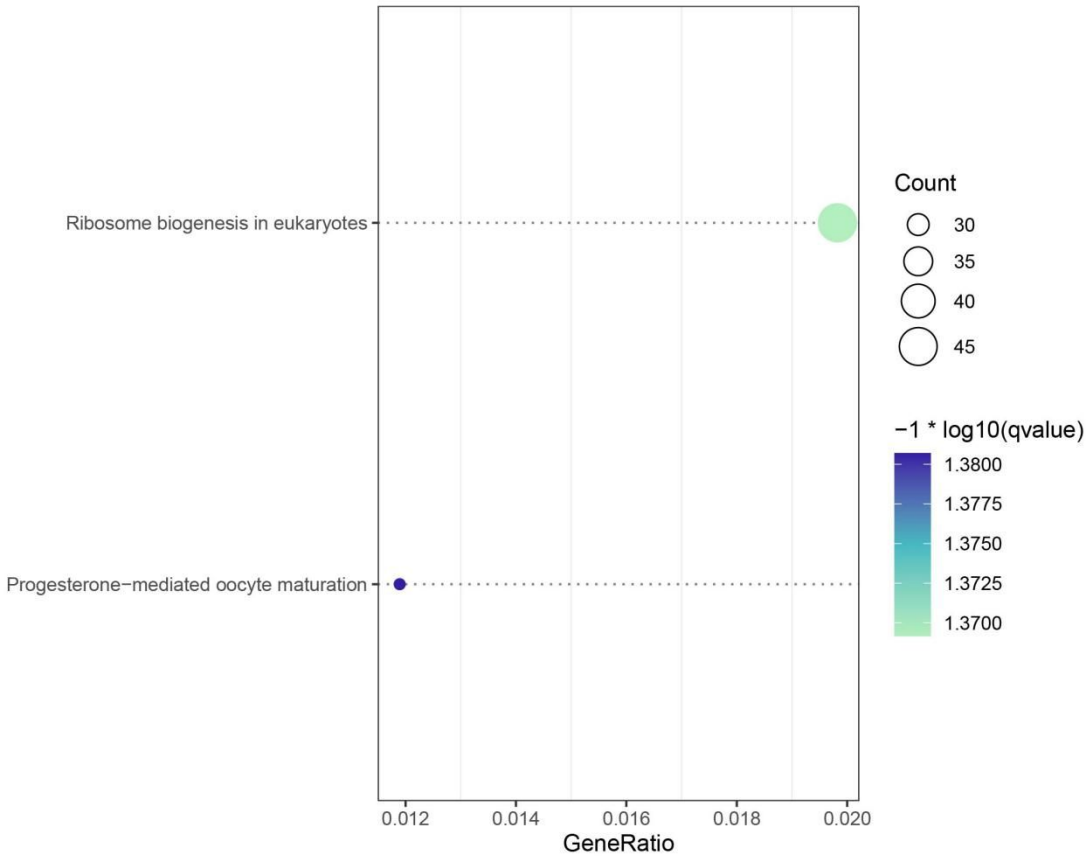

Figure 28. *The enriched KEGG terms of the Cluster 2 DEGs in IPC roots under salt treatment.*

The top 10 enriched KEGGs with Culster3 genes in roots are (Figure 29): Vascular smooth muscle contraction, Toxoplasmosis, Endocytosis, Toll and Imd signaling pathway, Insulin signaling pathway, Alcoholism, Endocrine and other factor-regulated calcium reabsorption, MAPK signaling pathway, Legionellosis, Influenza A.

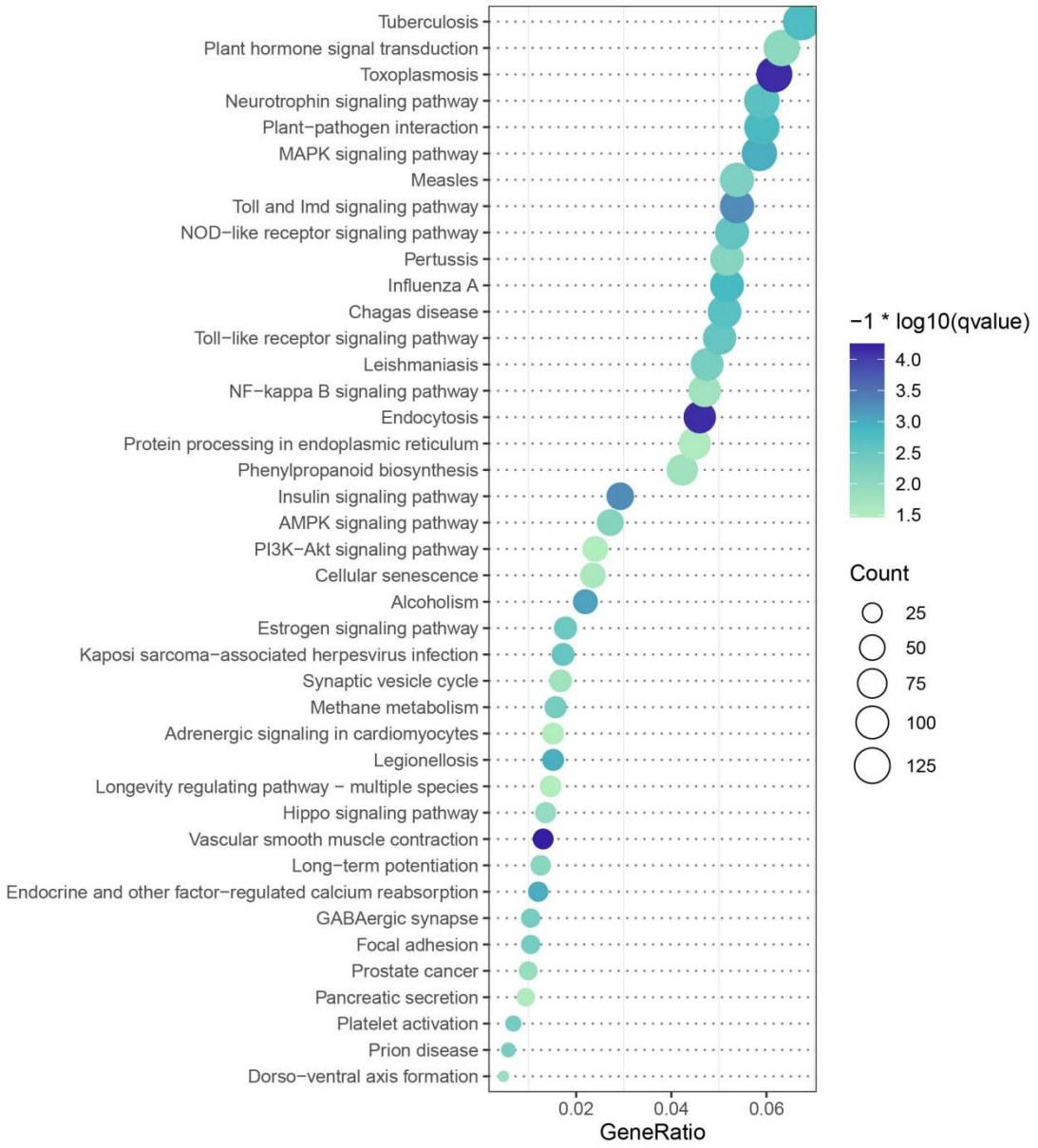

Figure 29. *The enriched KEGG terms of the Cluster 3 DEGs in IPC roots under salt treatment.*

The enriched KEGGs with Culster4 genes in roots are (Figure 30): Ribosome, Spliceosome, Proteasome, N-Glycan biosynthesis, Fanconi anemia pathway, Various types of N-glycan biosynthesis, DNA replication, Aminoacyl-tRNA biosynthesis.

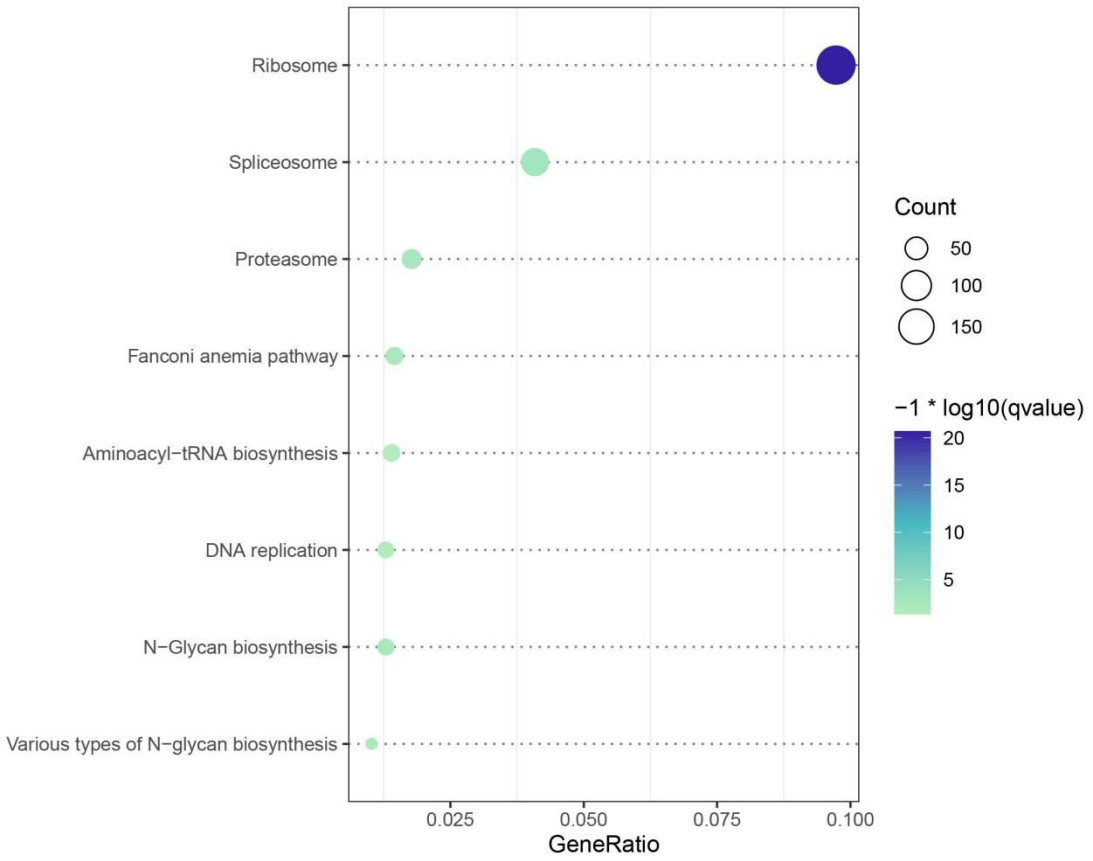

Figure 30. *The enriched KEGG terms of the Cluster 4 DEGs in IPC roots under salt treatment.*

4.3 GO enrichment with leaves

According to the gene expression in leaves, the DEGs are divided into four clusters. The number of genes in cluster 1, cluster 2, cluster 3, and cluster 4 are 7185, 7454, 6031, and 6790 (Figure 31):.

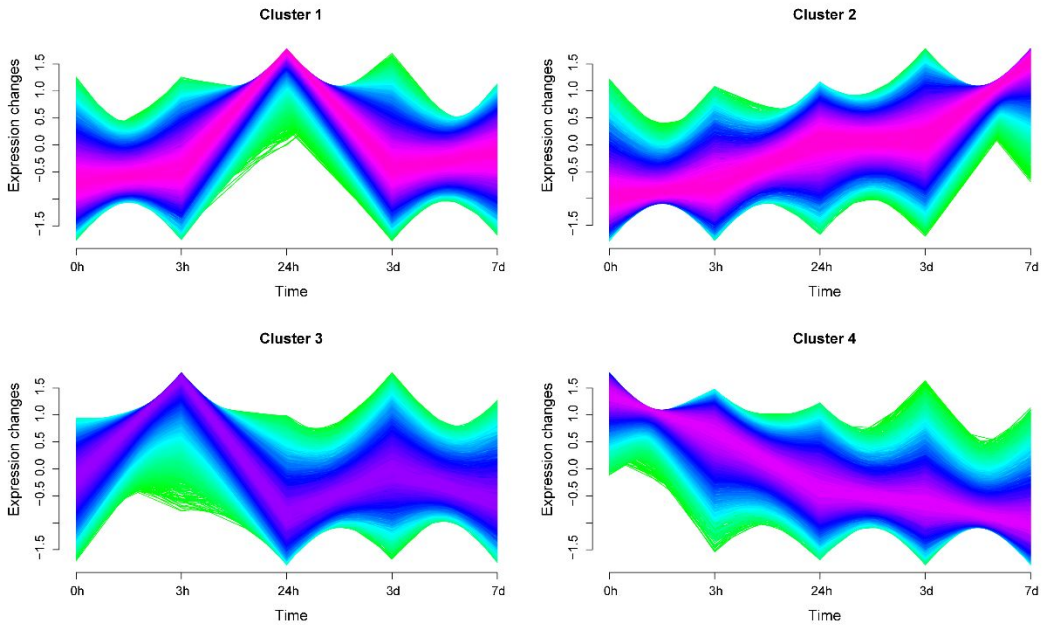

Figure 31. *Four gene clusters of DEGs in IPC leaves under salt treatment.*

GO enrichment analysis was conducted with four groups of genes in leaves.

The top 10 enriched GO with the Culster1 gene in leaves are (Figure 32): vesicle-mediated transport, cellular macromolecule localization, cellular protein localization, establishment of protein localization, intracellular protein transport, protein transport, peptide transport, amide transport, nucleocytoplasmic transport, nuclear transport.

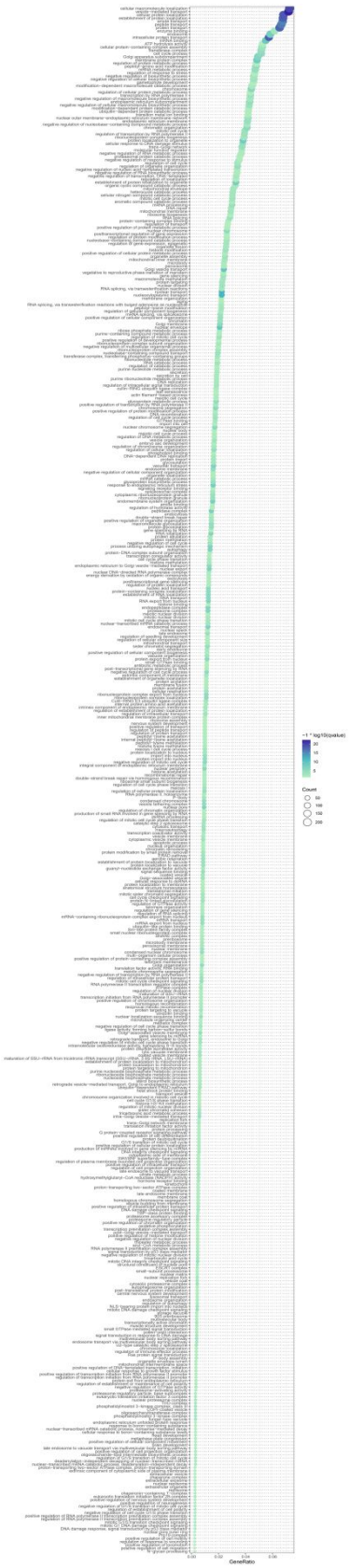

Figure 32. *The enriched GO terms of the Cluster 1 DEGs in IPC leaves under salt treatment.*

The top 10 enriched GO with Culster 2 gene in leaves are (Figure 33):

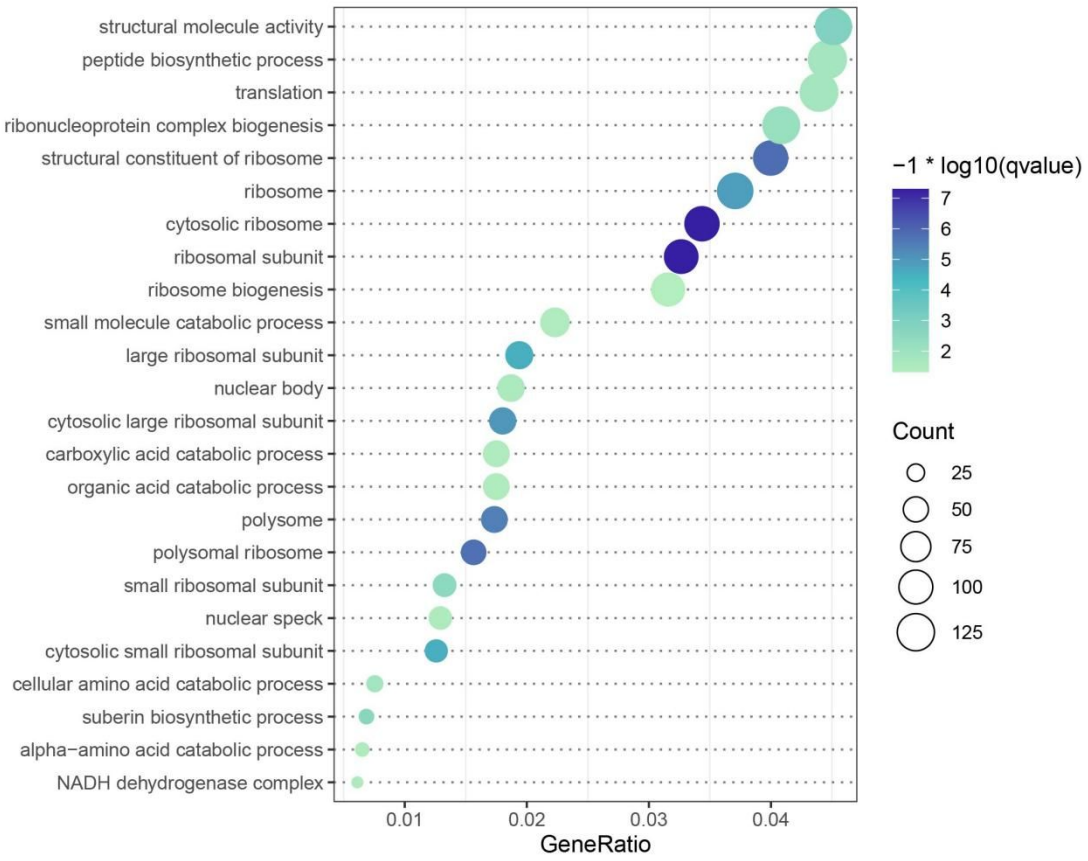

Figure 33. *The enriched GO terms of the Cluster 2 DEGs in IPC leaves under salt treatment.*

The top 10 enriched GO with Culster 3 gene in leaves are (Figure 34):

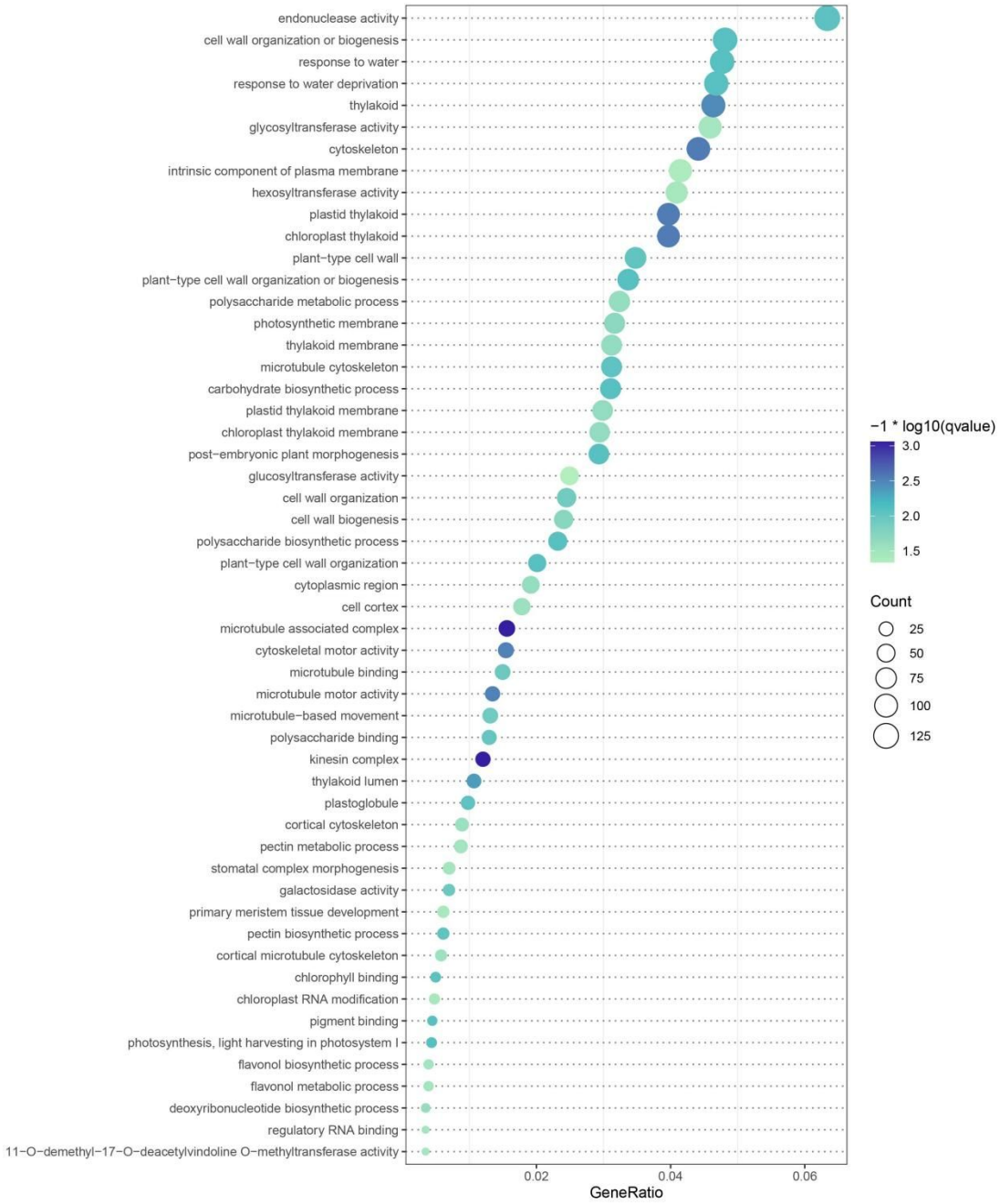

Figure 34. *The enriched GO terms of the Cluster 3 DEGs in IPC leaves under salt treatment.*

The top 10 enriched GO with Culster 4 gene in leaves are (Figure 35):

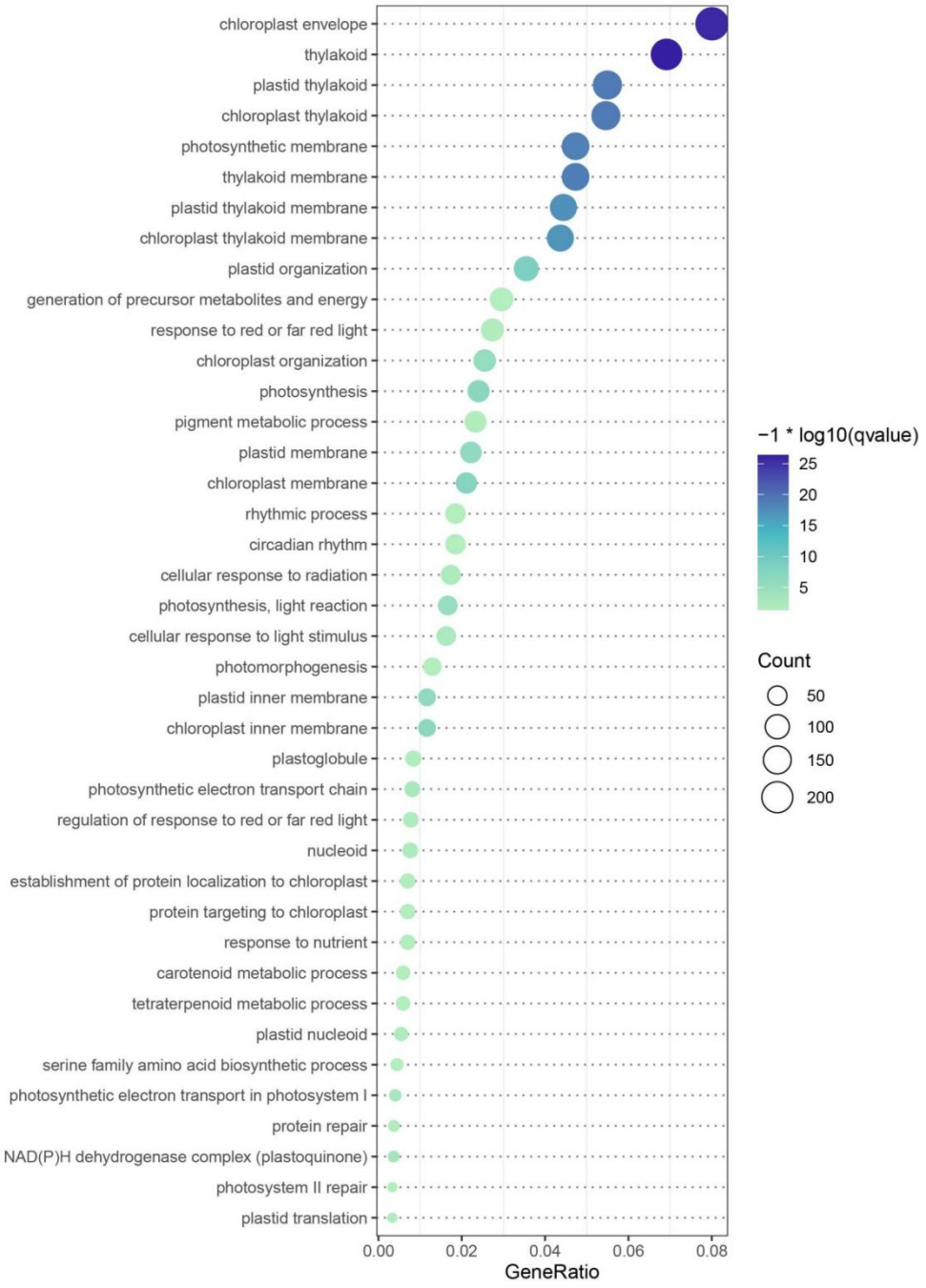

Figure 35. *The enriched GO terms of the Cluster 4 DEGs in IPC leaves under salt treatment.*

4.4 KEGG enrichment with leaves

KEGG enrichment analysis was conducted with four groups of genes in leaves.

The top 10 enriched KEGGs with Culster 1 genes in leaves are (Figure 36): Nucleocytoplasmic transport, Endocytosis, Proteasome, Protein processing in endoplasmic reticulum, N-Glycan biosynthesis, Human papillomavirus infection, mRNA surveillance pathway, Various types of N-glycan biosynthesis, Citrate cycle (TCA cycle), and Alzheimer disease.

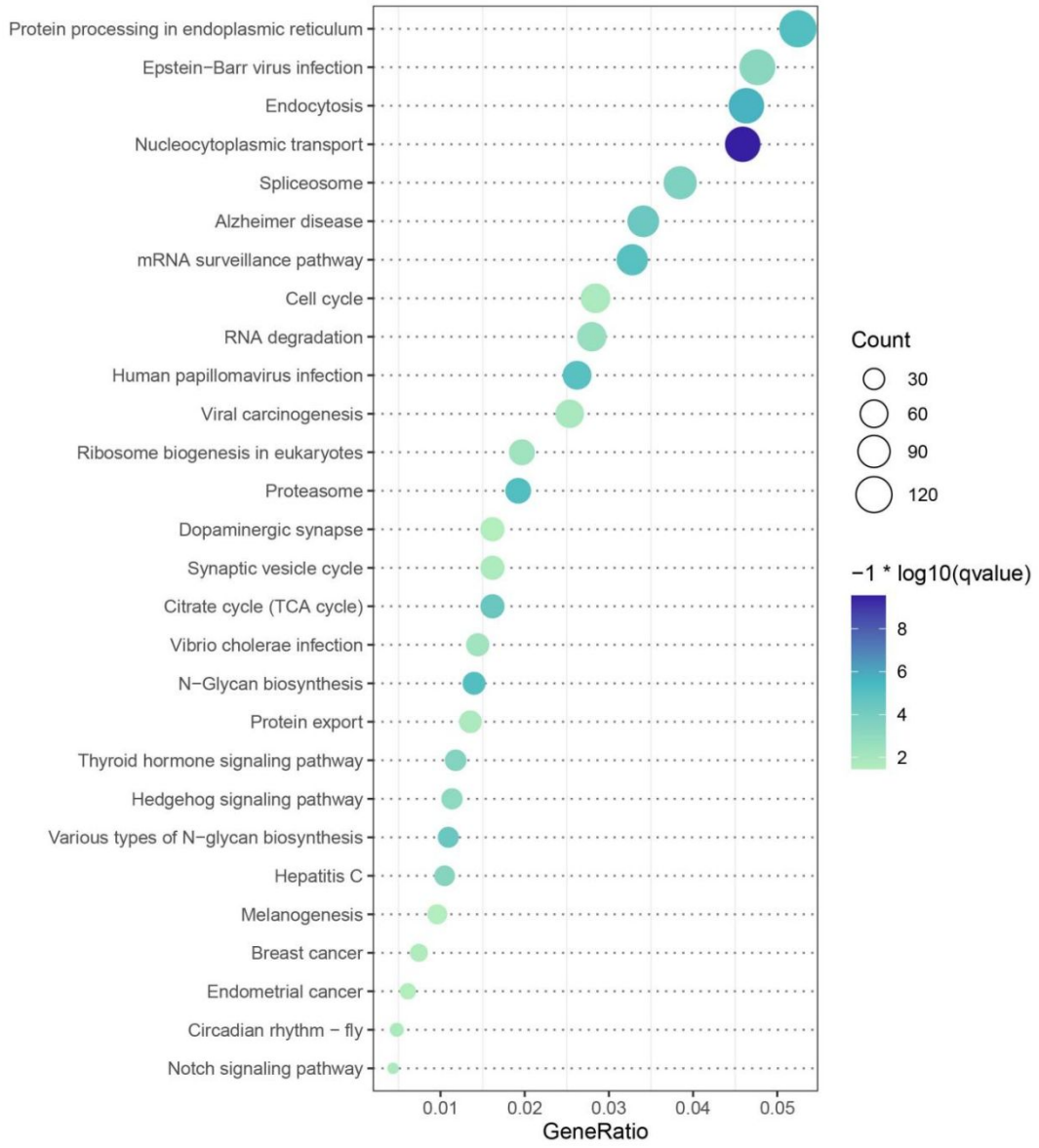

Figure 36. *The enriched KEGG terms of the Cluster 1 DEGs in IPC leaves under salt treatment.*

The enriched KEGGs with Culster2 genes in leaves are (Figure 37): Ribosome.

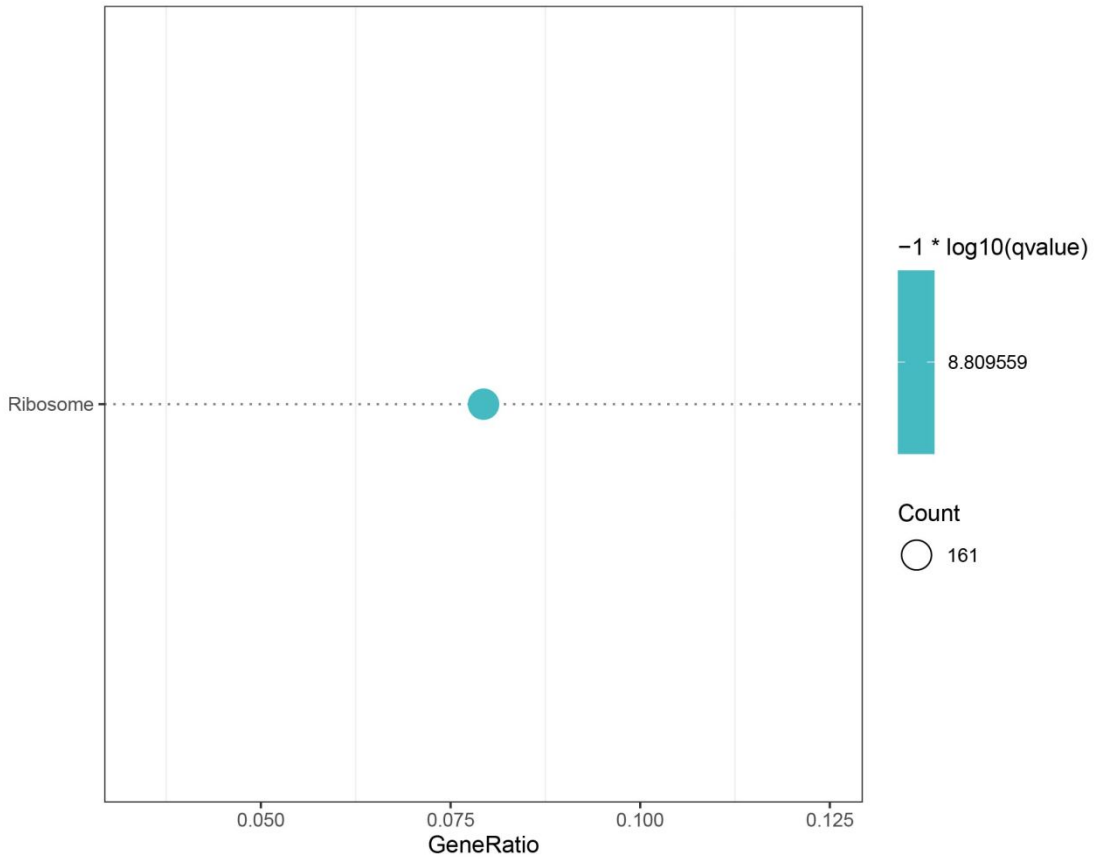

Figure 37. *The enriched KEGG terms of the Cluster 2 DEGs in IPC leaves under salt treatment.*

The enriched KEGGs with Culster3 genes in leaves are (Figure 38):

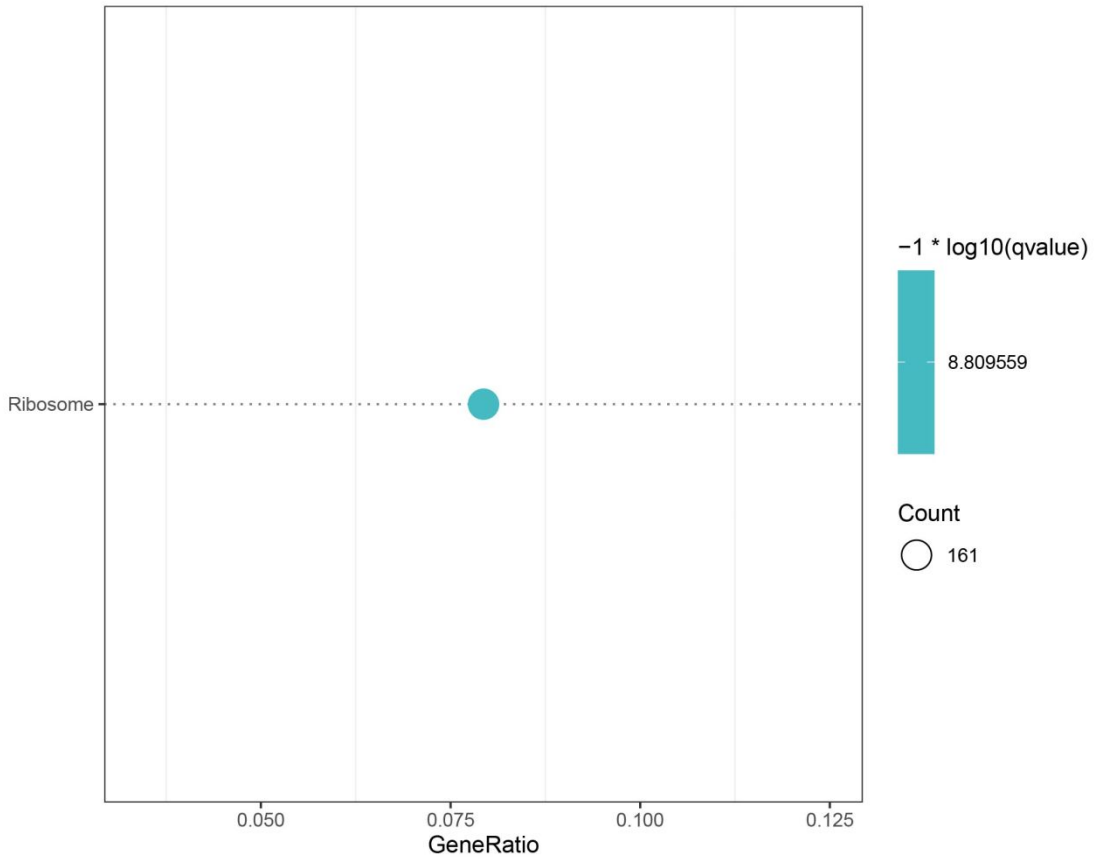

Figure 38. *The enriched KEGG terms of the Cluster 3 DEGs in IPC leaves under salt treatment.*

The enriched KEGGs with Cluster 4 genes in leaves are (Figure 39): Linoleic acid metabolism, Porphyrin metabolism, Chemical carcinogenesis – DNA adducts, Metabolism of xenobiotics by cytochrome P450, Riboflavin metabolism, and MAPK signaling pathway.

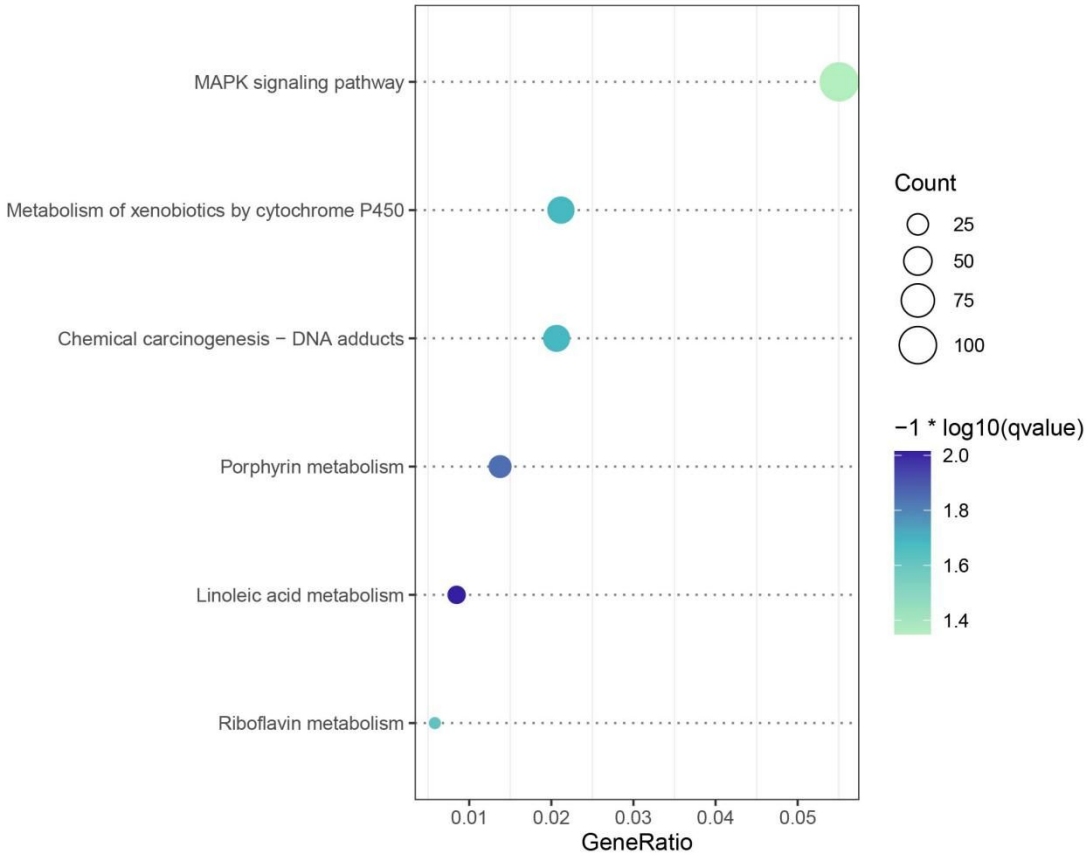

Figure 39. *The enriched KEGG terms of the Cluster 4 DEGs in IPC leaves under salt treatment.*

References

1. Akinniyi G, Lee J, Kim H, Lee J-G, Yang I. 2022. A medicinal halophyte Ipomoea pes-caprae (Linn.) r. br.: A review of its botany, traditional uses, phytochemistry, and bioactivity. *Marine Drugs* 20:329