

a SOD vitality (U/g)

Treatment	SOD vitality (U/g)
CK	~90 ^a
Cs	~45 ^c
NaCl	~60 ^b
Cs+NaCl	~70 ^b

b POD vitality (U/g)

Treatment	POD vitality (U/g)
CK	~135 ^a
Cs	~100 ^b
NaCl	~115 ^b
Cs+NaCl	~120 ^b

c CAT vitality (U/g)

Treatment	CAT vitality (U/g)
CK	~55 ^a
Cs	~58 ^a
NaCl	~72 ^a
Cs+NaCl	~55 ^b

d MDA content (nmol/g)

Treatment	MDA content (nmol/g)
CK	~2.8 ^a
Cs	~4.5 ^{ab}
NaCl	~3.8 ^a
Cs+NaCl	~3.3 ^b

e Soluble sugar (μmol/L)

Treatment	Soluble sugar (μmol/L)
CK	~1.6 ^a
Cs	~1.7 ^a
NaCl	~1.8 ^{ab}
Cs+NaCl	~2.0 ^a

f Soluble Protein Content (mg/g)

Treatment	Soluble Protein Content (mg/g)
CK	~22 ^{ab}
Cs	~18 ^a
NaCl	~20 ^a
Cs+NaCl	~22 ^a

g AsA content (nmol g⁻¹ FW)

Treatment	AsA content (nmol g ⁻¹ FW)
CK	~0.45 ^a
Cs	~0.28 ^b
NaCl	~0.32 ^b
Cs+NaCl	~0.43 ^a

h GSH content (nmol g⁻¹ FW)

Treatment	GSH content (nmol g ⁻¹ FW)
CK	~0.30 ^a
Cs	~0.38 ^a
NaCl	~0.50 ^a
Cs+NaCl	~0.55 ^a

i NPT content (nmol g⁻¹ FW)

Treatment	NPT content (nmol g ⁻¹ FW)
CK	~0.50 ^a
Cs	~0.20 ^a
NaCl	~0.18 ^b
Cs+NaCl	~0.38 ^a

j PCs content (nmol g⁻¹ FW)

Treatment	PCs content (nmol g ⁻¹ FW)
CK	~0.02 ^a
Cs	~0.18 ^a
NaCl	~0.35 ^a
Cs+NaCl	~0.30 ^a

a Cu accumulation (mg/kg) in leaves, shoots, and roots for CK, Cu, Cu+Ce, and Ce treatments. Cu accumulation is highest in leaves and shoots, with a significant increase in the Cu+Ce treatment compared to Cu alone.

b Ce accumulation (mg/kg) in leaves, shoots, and roots for CK, Cu, Cu+Ce, and Ce treatments. Ce accumulation is highest in leaves and shoots, with a significant increase in the Cu+Ce treatment compared to Cu alone.

c Subcellular distribution of Cu (mg/L) in soluble sub, cell membrane, and cell wall for CK, Cu, Cu+Ce, and Ce treatments. Cu is primarily located in the cell wall, with a significant increase in the Cu+Ce treatment compared to Cu alone.

d Subcellular distribution of Ce (mg/L) in soluble sub, cell membrane, and cell wall for CK, Cu, Cu+Ce, and Ce treatments. Ce is primarily located in the cell wall, with a significant increase in the Cu+Ce treatment compared to Cu alone.

e Venn diagrams showing the overlap of differentially expressed genes (DEGs) between CK vs Cu+Ce, CK vs Cu, and CK vs Ce treatments. The overlap between CK vs Cu+Ce and CK vs Cu is 108 (1.56%). The overlap between CK vs Cu+Ce and CK vs Ce is 208 (3.1%). The overlap between CK vs Cu and CK vs Ce is 64 (0.9%). The overlap between all three is 24 (0.36%).

f Bar chart showing the number of up-regulated (up) and down-regulated (down) DEGs for CK vs Cu+Ce, CK vs Cu, CK vs Ce, and CK vs Cu+Ce. The number of up-regulated DEGs is 317, 507, 511, and 360, respectively. The number of down-regulated DEGs is 240, 210, 458, and 280, respectively.

a

Number

MYB_superfamily
AP2/ERF
NAC
C2C2
bHLH
GRAS
WRKY
bZIP
C3H
B3_superfamily
LBD (AS1/CO1)
TCP
MADS
GRF
FAR1
HSR
C2H2
LOB
ZF-HD
SBP

b

MYB family

WRKY family

bHLH family

HSF family

bZIP family

NAC family

C2C2 family

AP2 family

TCP family

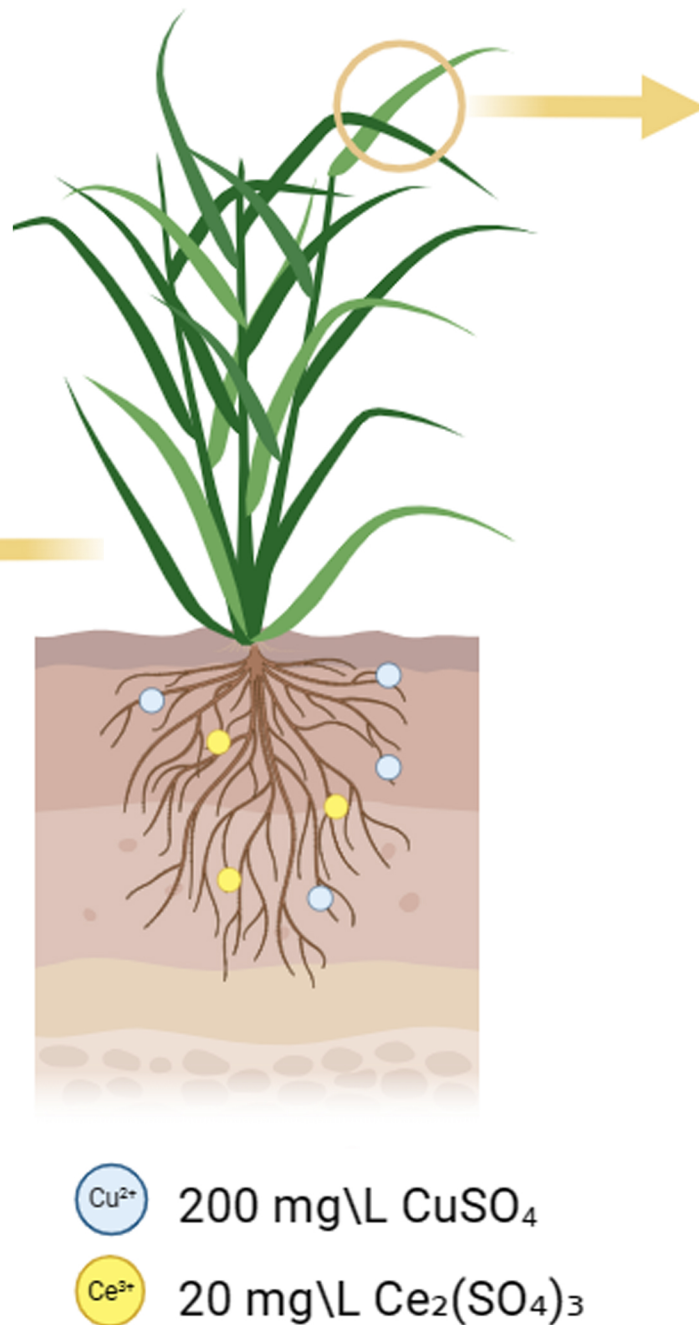
GRF family

C3H family

LOB family

ZF-HD family

0.00 0.50 1.00 1.50



a

KEGG pathways

Transport and catabolism

Replication and repair

Information processing in viruses

Transcription

Folding, sorting and degradation

Translation

Environmental adaptation

Membrane transport

Signal transduction

Glycan biosynthesis and metabolism

Metabolism of other amino acids

Metabolism of cofactors and vitamins

Nucleotide metabolism

Energy metabolism

Metabolism of terpenoids and polyketides

Global and overview maps

Lipid metabolism

Biosynthesis of other secondary metabolites

Amino acid metabolism

Carbohydrate metabolism

Number of genes

b

KEGG enrichment analysis(ck_vs_cu_G)

Isoprenoid alkaloid biosynthesis

Pentose

Flavonoid biosynthesis

Trypophan metabolism

Tropane, piperidine and pyridine alkaloid biosynthesis

Sequitripenoid and interpenoid biosynthesis

Glyoxylate and dicarboxylate metabolism

Biosynthesis of unsaturated fatty acids

Silibinin, diarylheptanoid and gingerol biosynthesis

Galactose metabolism

Phosphatidylinositol signaling system

Carotenoid biosynthesis

Purine metabolism

Circadian rhythm - plant

Cutin, suberine and wax biosynthesis

MAPK signaling pathway - plant

Starch and sucrose metabolism

Photosynthesis - antenna proteins

Phenylpropanoid biosynthesis

Plant-pathogen interaction

Rich Factor

c

KEGG enrichment analysis(ck_vs_cu_G)

AOC3 (DN1230)

N4OMT (DN5444)

cat (DN27190)

OMT (DN2431)

N4OMT (DN2431)

CP1 (DN19733)

galM (DN1523)

ACS (DN81246)

traA (DN1523)

FAR (DN6554)

CY84A (DN15062)

MKG9 (DN10428)

cat (DN2644)

cat (DN20473)

TR1 (DN10911)

sacA (DN3538)

CALM (DN6264)

GCSH (DN6229)

TME222 (DN2165)

SYM1 (DN11333)

scrK (DN2998)

CK CU Cb Cb

d

KEGG pathways

Environmental adaptation

Transport and catabolism

Information processing in viruses

Replication and repair

Transcription

Translation

Folding, sorting and degradation

Membrane transport

Signal transduction

Nucleotide metabolism

Metabolism of terpenoids and polyketides

Biosynthesis of other secondary metabolites

Metabolism of cofactors and vitamins

Global and overview maps

Lipid metabolism

Energy metabolism

Amino acid metabolism

Carbohydrate metabolism

Number of genes

e

KEGG enrichment analysis(cu_vs_cc_G)

N-Glycan biosynthesis

Silibinin, diarylheptanoid and gingerol biosynthesis

Isoprenoid alkaloid biosynthesis

Protein processing in endoplasmic reticulum

MAPK signaling pathway - plant

Flavonoid biosynthesis

Galactose metabolism

Various types of N-glycan biosynthesis

Photosynthesis - antenna proteins

Cysteine and methionine metabolism

Pyruvate metabolism

Plant hormone signal transduction

Glyoxylate and dicarboxylate metabolism

Penicillanic acid and CoA biosynthesis

Antimicrobial RNA biosynthesis

Limnane and pinene degradation

Fatty acid biosynthesis

ABC transporters

Tyrosine metabolism

Endocytosis

Rich Factor

f

KEGG enrichment analysis(cu_vs_cc_G)

RPS2 (DN81)

MDH1 (DN5326)

mtdm (DN559)

PP2C (DN469)

CK (DN5618)

WHY33 (DN3847)

CALM (DN23493)

HSP90A (DN23493)

ALDH (DN15285)

PDR (DN21092)

BZP1 (2 DN385)

PP2C (DN619)

N4OMT (DN5444)

OMT (DN2431)

RPS2 (DN27639)

ABG1 (DN54505)

ARK-A (DN3735)

ackA (DN5890)

adh (DN3279)

OMT (DN10215)

MDH1 (DN1201)

AOC3 (DN1230)

ABCB1 (DN5984)

GST (DN4608)

GST (DN4108)

GST (DN4549)

PP2C (DN4691)

PDR (DN29276)

CK CU Cb Cb

a Isoquinoline

Phenylalanine, tyrosine and tryptophan biosynthesis

Phenylalanine metabolism

4-Hydroxyphenylacetic acid

L-Tyrosine

Tyrosine

4-Methylumbelliferone

Dopamine

3,4-DHAP

(S) Norcoclaurine

N-Ornethyl-norcodine

Galanthamine

b Peroxisome

Receptor recycling

Membrane protein import

Matrix protein import

ROS metabolism

Peroxisomal division

ethersulpholipid biosynthesis

membrane proteins

antioxidant system

hydrogen peroxide metabolism (P151 type)

c MAPK signaling pathway

Pathogen infection

Phytohormones

Ethylene

Salt Drought/Osmotic stress

Abiotic stress

Dna2

H2O2

Ethylene synthesis

DNA

Stress responses

Wounding responses

Wounding

Ca²⁺

DNA

Maintainance the homeostasis of reactive oxygen species

d Phenylpropanoid Biosynthesis

Phenylalanine, tyrosine and tryptophan biosynthesis

Caffeoyl-CoA

5-Hydroxy-caffeoyl shikimate

Sinapoyl-CoA

5-Hydroxyferulic acid

Sinapoyl-malate

5-Hydroxy-p-coumaroyl lignin

Coumaroyl lignin

Sinapyl lignin

Hydroxy-phenyl lignin

e Plant Hormone Signal Transduction

Tryptophan metabolism

Cytokinin

DNA

Cell enlargement

Plant growth

Zeaxin biosynthesis

Cytokinin

DNA

Cell division

Shoot initiation

Carotenoid biosynthesis

Abscisic acid

DNA

Stomatal closure

Brassinosteroid

Brassinosteroid

DNA

Cell elongation

Cell division

Phenylalanine metabolism

Salicylic acid

DNA

Disease resistance

f Pyruvate metabolism

Glycolysis

Oxaloacetate

Lactate

Citrate cycle

Glyoxylate metabolism

Acetyl-CoA

Acetate hydro

Fatty acid biosynthesis

Biosynthesis of endoparasit antibiotics

Biosynthesis of type II polyketide backbone

Ethanol

The diagram illustrates the signaling pathways involved in copper (Cu) and cerium (Ce) toxicity in *Dendrobium nobile*. The plant is shown at the top, with Cu and Ce entering the system. Cu enters as Cu²⁺ and Ce enters as Ce³⁺. The pathways are as follows:

- Copper (Cu) Toxicity Pathway:**
 - Cu²⁺ enters the cell and can be up-regulated (red arrow) or down-regulated (blue arrow).
 - Cu²⁺ reacts with O₂ and OH to form H₂O₂.
 - H₂O₂ leads to ROS generation, oxidative DNA damage, and cat (catalase) activity.
 - Cat activity is up-regulated (red arrow) and leads to Peroxisome/MAKIP signaling pathway.
 - Cu²⁺ can also be down-regulated (blue arrow) by cat.
 - Cu²⁺ enters the vacuole via ABC transporters (indicated by a red X, suggesting inhibition or down-regulation).
 - In the vacuole, Cu²⁺ is reduced to Cu⁺ (reduce Cu²⁺), leading to stomatal closure and resistance.
 - Protein processing in the endoplasmic reticulum is affected by Cu.
- Cerium (Ce) Toxicity Pathway:**
 - Ce³⁺ enters the cell and can be up-regulated (red arrow) or down-regulated (blue arrow).
 - Ce³⁺ interacts with the cell wall and GST (Glutathione S-transferase) and Glutathione metabolism.
 - Ce³⁺ leads to AsA (Ascorbic acid) and GSH (Glutathione) levels, and NPT (Nucleoside Phosphate Transferase) and PCs (Phenyl Compounds).
 - Ce³⁺ leads to chelation (indicated by a yellow star) and PP2C (Protein Phosphatase 2C).
 - Chelation leads to ABA (Abscisic Acid) and Plant hormone signal transduction.
 - Chelation also leads to MYB78, ERF2, ERF9, ERF053, WRKY71, WRKY24, WRKY50, DHLH63, and DHLH41, which are Transcription Factors.
 - Chelation leads to resistance (indicated by a blue arrow).
- Antioxidant Defense:**
 - Antioxidant defense is up-regulated (red arrow) by ROS generation, oxidative DNA damage, and cat.
 - Antioxidant defense is down-regulated (blue arrow) by PDR (Plasma Membrane Divalent Ion Transporter).