

Supplemental material

Table S1. Relative fluorescence intensity in tall fescue roots. The fluorescence was quantified with ImageJ program and registered in fifteen squares of 1000 μm^2 each photo. Values are presented as a ration to the untreated Con (n = 4).

Treatment	SNP	c-PTIO	L-NAME	L-NAME +c-PTIO	Con	Cd	T1	T2
NO content	1.97	0.83	0.71	0.28	1.00	1.38	2.44	0.92

Table S2. Significance at Cd level in Table 1 analyzed by LSD test. The significant difference was presented as capital letters at $P < 0.01$ and small letters at $P < 0.05$, respectively.

Cd	Mean Xi	Significance	
		$\alpha=0.05$	$\alpha=0.01$
With Cd	1.58	a	A
Without Cd	1.08	b	B

Table S3. Significance at NO level in Table 1 analyzed by LSD test. The significant difference was presented as capital letters at $P < 0.01$ and small letters at $P < 0.05$, respectively.

NO	Mean Xi	Significance	
		$\alpha=0.05$	$\alpha=0.01$
SNP	1.58	a	A
Without NO addition [†]	1.08	b	B
L-NAME+c-PTIO	0.60	c	C

[†] “Without NO addition” refers to Control and Cd treatment

Table S4. Summary of sequence assembly after illumina sequencing.

Sample [†]	Raw Reads	Clean Reads	Clean Bases	Error (%)	Q20 (%)	Q30 (%)	GC (%)
Con1	62584984	60907468	9.14G	0.01	97.59	93.81	53.7
Con2	46259288	45480844	6.82G	0.01	97.32	93.16	50.37
Con3	53117390	51810710	7.77G	0.01	97.44	93.54	52.08
Cd1	58625108	57334986	8.6G	0.01	97.29	93.06	55.42
Cd2	49789754	48859078	7.33G	0.02	97.06	92.62	55.23
Cd3	52984272	51976104	7.8G	0.02	97.11	92.69	55.36
T11	48057290	47193350	7.08G	0.02	97.13	92.76	55.11
T12	54031232	52886932	7.93G	0.01	97.42	93.32	55.03
T13	50177600	49288134	7.39G	0.01	97.32	93.14	55.14
T21	55495822	54533056	8.18G	0.01	97.22	92.92	55.48
T22	46965256	46078954	6.91G	0.01	97.43	93.3	55.55
T23	59562528	58495636	8.77G	0.01	97.18	92.82	55.42

[†] The sample were from four treatment regimes in this study, including the control (Con), Cd treatment, T1 treatment, and T2 treatment, and each regime had three replicates. They respectively presented the tall fescue seedlings were cultivated in 1/2 Hoagland solution (control, Con), 1/2 Hoagland solution with 50 mg/L Cd²⁺ (CdCl₂•2.5H₂O) (Cd treatment), 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM SNP (T1 treatment), and 1/2 Hoagland solution with 50 mg/L Cd²⁺, 200 μM L-NAME and 100 μM c-PTIO (T2 treatment).

Table S5 Length distribution of the transcripts and unigenes clustered from the de novo assembly.

Category	Transcripts	Unigenes
200-500bp	1317122	705398
500-1kbp	472146	183636
1k-2kbp	181139	66912
>2kbp	35170	12978
Total	2005577	968924
N50	654	560
N90	258	241
max length	14405	14405
min length	201	201
mean length	534	475

Table S6. Different metabolite levels in T1 treatment and Cd treatment in tall fescue. The tall fescue seedlings were cultivated in 1/2 Hoagland solution with 50 mg/L Cd²⁺ (CdCl₂•2.5H₂O) (Cd treatment) and 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM SNP (T1 treatment), respectively. Each value is the mean of three replicates.

Compounds	Class	Fold.Change	type
L-Alanine	Amino acids	2.537444934	up
L-Citrulline	Amino acids	2.362606232	up
L-PyroglutamicAcid	Amino acids	4.923076923	up
L-Asparagine	Amino acids	2.12	up
L-Asparagine Anhydrous	Amino acids	2.357769974	up
Homocystine	Amino acids	0.390628707	down
(-)-3-(3,4-Dihydroxyphenyl)-2-methylane	Amino acid derivatives	2.008572149	up
1-Methylhistidine	Amino acid derivatives	3.295519002	up
D-Pipecolinic acid	Amino acid derivatives	2.006944444	up
L-Homocitrulline	Amino acid derivatives	2.06190823	up
N6-Acetyl-L-lysine	Amino acid derivatives	2.049333333	up
Lysine butyrate	Amino acid derivatives	2.68695979	up
Kinurenine	Amino acid derivatives	2.144266338	up
Quisqualic acid	Aminoacid derivatives	3.601286174	up
sn-Glycero-3-phosphocholine	Cholines	2.086795937	up
Coumaroyl choline	Cholines	0.423382046	down
epicatechingallate(ECG)	Catechin and its derivatives	2.489944212	up
(-)-Epicatechingallate	Catechin and its derivatives	2.342157594	up
(-)-catechingallate	Catechin and its derivatives	2.862821789	up
catechingallate(CG)	Catechin and its derivatives	2.152084102	up
N',N'',N'''-p-coumaroyl-cinnamoyl-caffeoylspermidine	Phenolamides	0.378830409	down
N-Feruloylspermidine	Phenolamides	0.453575482	down
N-p-coumaroylagmatine	Phenolamides	0.397212544	down
N-feruloylagmatine	Phenolamides	0.389864526	down
N-p-coumaroylagmatine iso2	Phenolamides	0.369649805	down
Putrescine derivative	Phenolamides	2.050096805	up
N-Caffeoylagmatine	Phenolamides	0.197477787	down
2'-Deoxycytidine-5'-diphosphate	Nucleotide and its derivates	2.731804586	up
2'-Deoxyguanosine	Nucleotide and its derivates	0.404396371	down
6-Benzylaminopurine	Nucleotide and its derivates	2.539940828	up
8-Hydroxyguanosine	Nucleotide and its derivates	0.494842233	down
Uracil	Nucleotide and its derivates	0.490692707	down
Delphinidin 3-Glu	Anthocyanin	2.996270203	up
Malvidin 3-O-galactoside	Anthocyanin	2.573048408	up
Peonidin	Anthocyanin	360.7037037	up
Peonidin O-hexoside	Anthocyanin	10.258972	up
Cyanidin O-syringic acid	Anthocyanin	4.939682423	up
Cyanidin O-hexosyl-O-hexosyl-O-hexoside	Anthocyanin	3.147249191	up
KUROMANIN CHLORIDE	Anthocyanin	6.103195995	up

Cyanidin 3-galactoside (Idaein chloride)	Anthocyanin	5.868323816	up
Cyanidin 3-glucoside	Anthocyanin	6.109303839	up
Cyanidin 3-rutinoside (Keracyanin)	Anthocyanin	4.205819156	up
Rosinidin 3-O-glucoside	Anthocyanin	2.207259953	up
3,7-Dihydroxy-3',4'-dimethoxyflavone	Flavone	0.467307692	down
Acacetin	Flavone	0.000417195	down
Chrysoeriol 7-O-hexoside	Flavone	3.077022112	up
Chrysoeriol 7-O-rutinoside	Flavone	3.699262546	up
Chrysoeriol O-malonylhexoside	Flavone	3.013590703	up
Chrysoeriol O-hexoside	Flavone	2.255828319	up
Chrysoeriol O-rhamnosyl-O-hexoside	Flavone	5.205128205	up
Chrysoeriol 5-O-hexoside	Flavone	3.685714286	up
Tricin 5-O-hexoside	Flavone	2.292136164	up
Tricin 5-O-hexosyl-O-hexoside	Flavone	5.016227444	up
Tricin 7-O-hexoside	Flavone	2.390762289	up
Luteolin O-rutinoside	Flavone	0.099524946	down
Apigenin O-malonylhexoside	Flavone	5.238995693	up
Avicularin	Flavone	0.007969303	down
Rhoifolin	Flavone	99.92592593	up
Rhoifolin (Apigenin7-O-neohesperidoside)	Flavone	0.075325981	down
Isorhoifolin	Flavone	0.118077297	down
sakuranetin	Flavone	0.000318329	down
C-hexosyl-apigenin O-hexoside	Flavonoids-C	0.111496113	down
C-hexosyl-apigenin O-p-coumaroylhexoside	Flavonoids-C	0.001139241	down
Vitexin 2''-O-beta-L-rhamnoside	Flavonoids-C	2.51641791	up
Quercetin-3-(6''-malonyl)-Glucoside	Flavonol	0.393769866	down
Kaempferol 3-O-robinoside (Biorobin)	Flavonol	0.108374172	down
Kaempferol-3-Glucoside-6''-p-coumaroyl	Flavonol	0.180242311	down
Kaempferitrin (Kaempferol 3,7-dirhamnoside)	Flavonol	0.181373744	down
1-O-caffeoylquinic acid	Quinate and its derivatives	3.201612903	up
4'-Hydroxy-5,7-dimethoxyflavanone	Flavonoids	2.593309859	up
3,4',5,7-tetrahydroxy-3'-methoxyflavone	Flavonoids	2.01627907	up
2,5-Dihydroxybenzoate	Others	0.491635688	down
2,6-Xylidine	Others	2.017184943	up
2-Amino-1,3-octadecanediol	Others	2.822037878	up
8-Chlro-1-tetrahydronorharmanone	Others	2.094071806	up
alpha-(3,4-dihydroxyphenyl)lactic acid	Others	3.660508083	up
Phloridzin	Others	3.089959373	up
Xanthoxylin	Others	0.360176391	down
Phellodenol H	Others	2.808445129	up
12OHJA-Ile-1	Others	2.297953356	up
Carnitine	Others	2.744807122	up
Griseofulvin	Others2	0.491253644	down
sesamin	Others2	2.234042553	up

1-O-b-D-glucopyranosyl sinapate	Hydroxycinnamoyl derivatives	0.255670103	down
Disinapoylhexoside	Hydroxycinnamoyl derivatives	2.663120567	up
trans-Cinnamic acid	Hydroxycinnamoyl derivatives	2.705971277	up
Pipecolate	Alkaloids	2.346207865	up
2-Propenyl (sinigrin)	Carbohydrates	0.253641618	down
D(-)-Threose	Carbohydrates	0.322994652	down
Ribitol	Carbohydrates	3.53649635	up
Cafestol	Terpenoids	2.039835339	up
Oryzalexin?C	Terpenoids	2.191019648	up
6-hydroxynicotinic acid	Nicotinic acid and its derivatives	0.294462901	down
2-Picolinic acid	Organic acid and its derivatives	0.49	down
2-furanoic acid	Organic acid and its derivatives	2.131595282	up
DL-Pipecolinic acid	Organic acid and its derivatives	2.163321799	up
Quisqualate	Organic acid and its derivatives	0.461415306	down
13-HPODE	Lipids-fatty acid	0.393536122	down
3-Indolebutyric acid	Phytohormones	4.078031809	up

Table S7 The fold changes of selected metabolites in T1 vs Cd comparison[†]

Compounds	Class	Fold. Change [†]
Peonidin	Anthocyanin	360.7037
Rhoifolin	Flavone	99.92593
Peonidin O-hexoside	Anthocyanin	10.25897
Cyanidin 3-glucoside	Anthocyanin	6.109304
KUROMANIN CHLORIDE	Anthocyanin	6.103196
Avicularin	Flavone	0.007969
C-hexosyl-apigenin	Flavonoids-C	0.001139
O-p-coumaroylhexoside		
Acacetin	Flavone	0.000417
Sakuranetin	Flavone	0.000318

[†]The tall fescue seedlings were cultivated in 1/2 Hoagland solution with 50 mg/L Cd²⁺ (CdCl₂•2.5H₂O) (Cd treatment) and 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM SNP (T1 treatment), respectively. Each value is the mean of three replicates. Refer to the ratio of the concentration of metabolites of T1 treatment to the concentration of metabolites of Cd treatment.

Table S8. The level of related DEGs and different metabolites response to T1 treatment vs Cd treatment. The tall fescue seedlings were cultivated in 1/2 Hoagland solution with 50 mg/L Cd²⁺ (CdCl₂•2.5H₂O) (Cd treatment) and 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM SNP (T1 treatment), respectively. Each value is the mean of three replicates.

Term	Compounds	Class	Fold.Change (SNPvsCd)	Fold.Change (PLvsCd)	Gene_id	Description	log2ratio(SNPvs Cd)	log2ratio(P LvsCd)
ABC transporters	Carnitine	Others	2.7448	0.5292	TRINITY_DN 400262_c7_g2	ATP-binding cassette, subfamily B (MDR/TAP), member 1	1.5112	0.60218
					TRINITY_DN 370337_c1_g4	ATP-binding cassette, subfamily B (MDR/TAP), member 1	1.9914	1.2374
					TRINITY_DN 367086_c1_g2	ATP-binding cassette, subfamily C (CFTR/MRP), member 10	2.8355	2.1535
Phenylpropanoid biosynthesis	trans-Cinnamic acid	Hydroxycinnamoyl derivatives	2.7060	1.4187	TRINITY_DN 384579_c0_g2	peroxidase	-1.833	-0.8813
					TRINITY_DN	peroxidase	-2.1992	-0.7401

					338664_c3_g1				
					TRINITY_DN	trans-cinnamate 4-monooxygenase	2.5086	2.0206	
					367754_c1_g1				
					TRINITY_DN	cinnamoyl-CoA reductase	3.4356	1.2925	
					345973_c0_g1				
					TRINITY_DN	peroxidase	-1.8802	-0.2061	
					386671_c2_g5				
					TRINITY_DN	trans-cinnamate 4-monooxygenase	2.7516	1.9301	
					359843_c1_g1				
					TRINITY_DN	ferulate-5-hydroxylase	-1.3505	-0.4690	
					400412_c2_g2				
					TRINITY_DN	trans-cinnamate 4-monooxygenase	2.3034	1.7221	
					359843_c2_g1				
					TRINITY_DN	trans-cinnamate 4-monooxygenase	2.8636	2.0238	
					380055_c1_g4				
					TRINITY_DN	trans-cinnamate 4-monooxygenase	2.286	1.7238	
					367872_c0_g4				
					TRINITY_DN	peroxidase	-1.8577	-0.1923	
					351829_c0_g4				
					TRINITY_DN	trans-cinnamate 4-monooxygenase	2.4327	1.8606	
					380055_c1_g2				
Phenylalanine metabolism	trans-Cinnamic acid	Hydroxycinnamoyl derivatives	2.7060	1.4187	TRINITY_DN	trans-cinnamate 4-monooxygenase	2.7516	1.9301	
					359843_c1_g1				

					TRINITY_DN 359843_c2_g1	trans-cinnamate 4-monooxygenase	2.3034	1.7221
					TRINITY_DN 380055_c1_g4	trans-cinnamate 4-monooxygenase	2.8636	2.0238
					TRINITY_DN 367872_c0_g4	trans-cinnamate 4-monooxygenase	2.286	1.7238
					TRINITY_DN 367754_c1_g1	trans-cinnamate 4-monooxygenase	2.5086	2.0206
					TRINITY_DN 380055_c1_g2	trans-cinnamate 4-monooxygenase	2.4327	1.8606
Flavone and flavonol biosynthesis	Isotrifoliin	Flavone	0.1181	4.5783	TRINITY_DN 386812_c0_g3	flavonoid 3',5'-hydroxylase	-1.7957	-0.5861
	Acacetin	Flavone	0.0004	0.3997				
Tyrosine metabolism	2,5-Dihydroxybenzoate/2,5-dihydroxybenzoic acid	Organic acid and its derivatives	0.4916	1.5613	TRINITY_DN 330049_c1_g1	alcohol dehydrogenase class-P	3.1165	0.4230
					TRINITY_DN 351047_c0_g3	alcohol dehydrogenase class-P	3.8692	2.3934
					TRINITY_DN 351303_c1_g2	alcohol dehydrogenase class-P	2.6383	1.5694

TRINITY_DN 336653_c1_g1	alcohol dehydrogenase class-P	2.2926	1.0464
TRINITY_DN 327493_c0_g1	alcohol dehydrogenase class-P	2.1846	1.3623
TRINITY_DN 346637_c0_g2	alcohol dehydrogenase class-P	2.1097	1.8310
TRINITY_DN 346637_c0_g1	alcohol dehydrogenase class-P	2.4206	1.5578
TRINITY_DN 330791_c2_g1	alcohol dehydrogenase class-P	2.9909	0.4736
TRINITY_DN 327493_c0_g2	alcohol dehydrogenase class-P	2.117	1.4849
TRINITY_DN 332185_c0_g2	alcohol dehydrogenase class-P	2.2426	1.0971
TRINITY_DN 330791_c2_g2	alcohol dehydrogenase class-P	2.0264	1.3224
TRINITY_DN 330791_c2_g6	alcohol dehydrogenase class-P	2.684	1.4922

Nitrogen metabolism	L-Asparagine	Amino acids	2.1200	1.0940	TRINITY_DN 399931_c4_g2	alcohol dehydrogenase class-P	2.0172	1.2775
					TRINITY_DN 336653_c1_g2	alcohol dehydrogenase class-P	2.2498	1.2887
					TRINITY_DN 379442_c0_g2	alcohol dehydrogenase class-P	1.7648	1.7195
					TRINITY_DN 380554_c0_g2	nitrate reductase (NAD(P)H)	2.6907	0.0345
					TRINITY_DN 380554_c0_g1	nitrate reductase (NAD(P)H)	2.5907	0.3174
					TRINITY_DN 348303_c1_g2	MFS transporter, NNP family, nitrate/nitrite transporter	-1.5092	-0.3867
					TRINITY_DN 348032_c3_g1	nitrate reductase (NAD(P)H)	1.8839	-0.3130
TRINITY_DN 353219_c1_g1	MFS transporter, NNP family, nitrate/nitrite transporter	-1.5166	-0.4028					

					TRINITY_DN 338027_c1_g1	glutamate synthase (NADPH/NADH)	1.9273	1.2264
					TRINITY_DN 396249_c7_g2	ferredoxin-nitrite reductase	1.4373	0.8337
					TRINITY_DN 367739_c0_g2	nitrate reductase (NAD(P)H)	2.2001	-0.0186
Arginine and proline metabolism	L-Citrulline	Amino acids	2.3626	1.6686	TRINITY_DN 346407_c0_g2	ornithine decarboxylase	-3.8219	-0.2918
					TRINITY_DN 346407_c0_g3	ornithine decarboxylase	-3.6495	-0.2479
					TRINITY_DN 325236_c8_g1	arginase	-2.5241	-1.0993
					TRINITY_DN 334409_c0_g1	ornithine decarboxylase	-3.0831	-0.9593
					TRINITY_DN 384769_c1_g2	prolyl 4-hydroxylase	2.0199	1.8756
					TRINITY_DN 384769_c1_g1	prolyl 4-hydroxylase	2.0408	1.8226
Glutathione metabolism	L-Pyroglutamic acid	Amino acids	4.9231	0.7554	TRINITY_DN 346407_c0_g2	ornithine decarboxylase	-3.8219	-0.2918

					TRINITY_DN 346407_c0_g3	ornithine decarboxylase	-3.6495	-0.2479
					TRINITY_DN 314196_c1_g1	glutathione S-transferase	Inf	NA
					TRINITY_DN 334409_c0_g1	ornithine decarboxylase	-3.0831	-0.9593
					TRINITY_DN 310547_c0_g1	glucose-6-phosphate 1-dehydrogenase	-4.4403	-2.7866
					TRINITY_DN 384370_c2_g1	glutathione S-transferase	3.8466	0.3196
					TRINITY_DN 358027_c0_g3	glutathione S-transferase	1.4609	0.8131
					TRINITY_DN 348788_c0_g1	glutathione S-transferase	1.7319	2.0212
Alanine, aspartate and glutamate metabolism	L-Asparagine	Amino acids	2.1200	1.0940	TRINITY_DN 339215_c0_g1	glutamate decarboxylase	2.2886	0.3310
	L-Alanine	Amino acids	2.5374	1.4626	TRINITY_DN 399803_c3_g7	glutamate decarboxylase	2.3775	0.3353
					TRINITY_DN 353982_c0_g1	glutamate decarboxylase	2.4279	0.6460

					TRINITY_DN 338027_c1_g1	glutamate synthase (NADPH/NADH)	1.9273	1.2264
Taurine and hypotaurine metabolism	L-Alanine	Amino acids	2.5374	1.4626	TRINITY_DN 339215_c0_g1	glutamate decarboxylase	2.2886	0.3310
					TRINITY_DN 399803_c3_g7	glutamate decarboxylase	2.3775	0.3353
					TRINITY_DN 353982_c0_g1	glutamate decarboxylase	2.4279	0.6460
					TRINITY_DN 400526_c2_g1	cysteamine dioxygenase	1.6843	0.8041
Carbon fixation in photosynthetic organisms	L-Alanine	Amino acids	2.5374	1.4626	TRINITY_DN 375458_c1_g1	malate dehydrogenase (NADP+)	-1.7817	-0.8755
					TRINITY_DN 385846_c1_g3	triosephosphate isomerase (TIM)	3.3106	1.9065
					TRINITY_DN 400305_c8_g2	pyruvate, orthophosphate dikinase	1.9903	1.0770

Cysteine and methionine metabolism					TRINITY_DN 272308_c0_g1	glyceraldehyde 3-phosphate dehydrogenase	-3.0477	-0.8655
					TRINITY_DN 385846_c1_g4	triosephosphate isomerase (TIM)	2.448	1.6071
	Homocystine	Amino acids	0.3906	0.3550	TRINITY_DN 383055_c1_g1	aspartate kinase	1.8368	1.0351
	L-Alanine	Amino acids	2.5374	1.4626	TRINITY_DN 367731_c3_g1	L-3-cyanoalanine synthase/ cysteine synthase	2.6683	-0.1042
					TRINITY_DN 383524_c1_g2	aspartate kinase	1.7086	1.0375
					TRINITY_DN 366863_c0_g2	DNA (cytosine-5)-methyltransferase 1	4.1621	2.9726
					TRINITY_DN 365736_c2_g1	S-adenosylmethionine synthetase 1	-2.4543	-1.0510
					TRINITY_DN 339974_c0_g1	serine O-acetyltransferase	0.01597	1.0000
					TRINITY_DN 319764_c1_g1	cystathionine beta-synthase	-2.9947	-1.0426

					TRINITY_DN 385145_c0_g2	L-3-cyanoalanine synthase/ cysteine synthase	3.6006	0.7211
					TRINITY_DN 352327_c0_g1	L-3-cyanoalanine synthase/ cysteine synthase	2.183	-0.4828
					TRINITY_DN 319404_c6_g2	adenosylhomocysteinase	-2.581	-0.9434
					TRINITY_DN 316068_c0_g1	L-3-cyanoalanine synthase/ cysteine synthase	2.5065	-0.0870
					TRINITY_DN 385145_c1_g1	L-3-cyanoalanine synthase/ cysteine synthase	2.2478	-0.1961
					TRINITY_DN 385145_c1_g3	L-3-cyanoalanine synthase/ cysteine synthase	2.5624	-0.1762
					TRINITY_DN 385145_c1_g2	L-3-cyanoalanine synthase/ cysteine synthase	2.6311	0.0577
beta-Alanine metabolism	Uracil	Nucleotide and its derivates	0.4907	0.2527	TRINITY_DN 339215_c0_g1	glutamate decarboxylase	2.2886	0.3310
					TRINITY_DN 399803_c3_g7	glutamate decarboxylase	2.3775	0.3353

					TRINITY_DN 353982_c0_g1	glutamate decarboxylase	2.4279	0.6460
Histidine metabolism	1-Methylhistidine	Amino acid derivatives	3.2955	1.1997	TRINITY_DN 338271_c6_g1	histidine decarboxylase	-3.2732	-2.0874
Pentose and glucuronate interconversions	Ribitol	Carbohydrates	3.5365	1.7555	TRINITY_DN 382339_c1_g6	UTP--glucose-1-phosphate uridylyltransferase	-2.6751	-1.2040
Purine metabolism	2'-Deoxyguanosine	Nucleotide and its derivates	0.4044	0.3217	TRINITY_DN 291157_c0_g1	nucleoside-diphosphate kinase	-2.4589	-1.0413
					TRINITY_DN 317143_c11_g 1	adenosine kinase	-2.6611	-1.0848
Aminoacyl-tRNA biosynthesis	L-Asparagine	Amino acids	2.1200	1.0940	TRINITY_DN 355582_c2_g3	glutaminyl-tRNA synthetase	-4.6101	-0.7478
	L-Alanine	Amino acids	2.5374	1.4626	TRINITY_DN 298425_c0_g2	seryl-tRNA synthetase	-2.9233	-1.4806
					TRINITY_DN 298319_c0_g1	valyl-tRNA synthetase	-3.8694	-0.4066

Cyanoamino acid metabolism	L-Asparagine	Amino acids	2.1200	1.0940	TRINITY_DN385145_c0_g2	L-3-cyanoalanine synthase/ cysteine synthase	3.6006	0.72108
					TRINITY_DN367731_c3_g1	L-3-cyanoalanine synthase/ cysteine synthase	2.6683	-0.1042
					TRINITY_DN352327_c0_g1	L-3-cyanoalanine synthase/ cysteine synthase	2.183	-0.48275
					TRINITY_DN316068_c0_g1	L-3-cyanoalanine synthase/ cysteine synthase	2.5065	-0.087038
					TRINITY_DN385145_c1_g1	L-3-cyanoalanine synthase/ cysteine synthase	2.2478	-0.19614
					TRINITY_DN385145_c1_g3	L-3-cyanoalanine synthase/ cysteine synthase	2.5624	-0.17621

					TRINITY_DN 385145_c1_g2	L-3-cyanoalanine synthase/ cysteine synthase	2.6311	0.057714
Pyrimidine metabolism	Uracil	Nucleotide and its derivates	0.4907	0.2527	TRINITY_DN 291157_c0_g1	nucleoside-diphosphate kinase	-2.4589	-1.0413
					TRINITY_DN 327045_c0_g1	dihydroorotate dehydrogenase	#NAME?	-6.2681
Glycerophospholipi d metabolism	sn-Glycero-3-phosph ocholine	Cholines	2.0868	0.7719	TRINITY_DN 334074_c3_g1	phosphatidylserine synthase 2	-3.4202	-1.2589

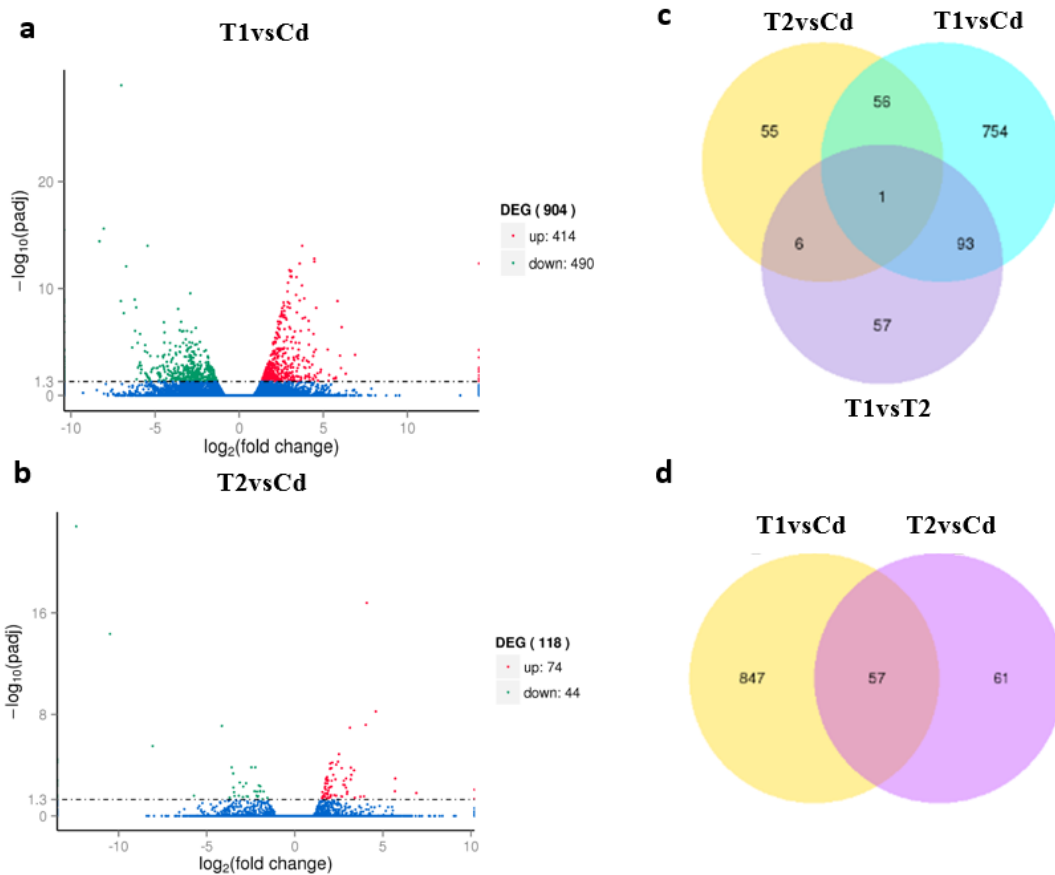


Fig. S1. Volcano Plots and Venn diagrams of significantly differentially expressed transcripts in the tall fescue roots with or without NO treatment under cadmium stress. (a) Volcano plot in T1vsCd. (b) volcano plot in T2vsCd. (c) Venn diagram analysis in different treatment. (d) Venn diagram analysis between T1vsCd and T2vsCd. Numbers indicate the number of transcripts with significant changes in expression under different conditions. Overlaps indicate the number of common transcripts differentially expressed, and numbers outside overlaps indicate the number of cultivar or subgroup specific transcripts differentially expressed. There were three regime, comprising Cd, T1, and T2. They respectively presented the tall fescue seedlings were cultivated in 1/2 Hoagland solution with 50 mg/L Cd^{2+} ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$) (Cd treatment), 1/2 Hoagland solution with 50 mg/L Cd^{2+} and 200 μM SNP (T1 treatment) and 1/2 Hoagland solution with 50 mg/L Cd^{2+} , 200 μM L-NAME and 100 μM c-PTIO (T2 treatment). Each value is the mean of three replicates.

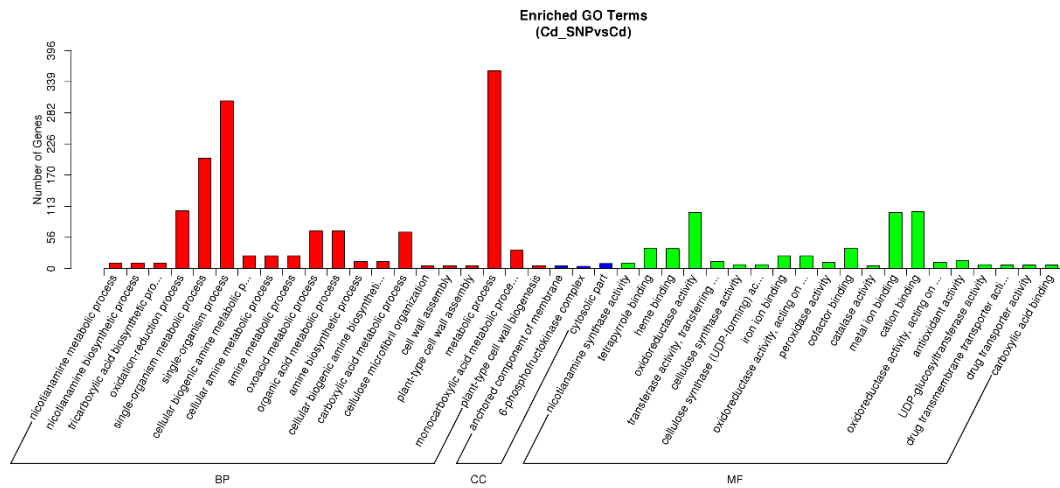


Fig. S2 Histogram of the gene ontology classification analysis of the DEGs in response to the T1 treatment in tall fescue roots. The tall fescue seedlings were cultivated in 1/2 Hoagland solution with 50 mg/L Cd²⁺ (CdCl₂•2.5H₂O) (Cd treatment) and 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM SNP (T1 treatment), respectively. Each value is the mean of three replicates.

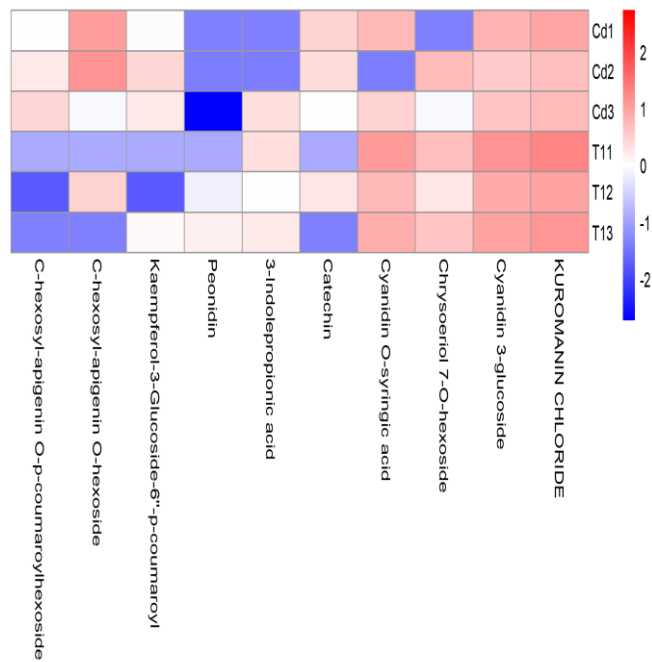


Fig. S3. The top 10 metabolites according to the VIP values in tall fescue under T1 treatment. The tall fescue seedlings were cultivated in 1/2 Hoagland solution with 50 mg/L Cd²⁺ (CdCl₂•2.5H₂O) (Cd treatment) and 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM SNP (T1 treatment), respectively. Each value is the mean of three replicates.

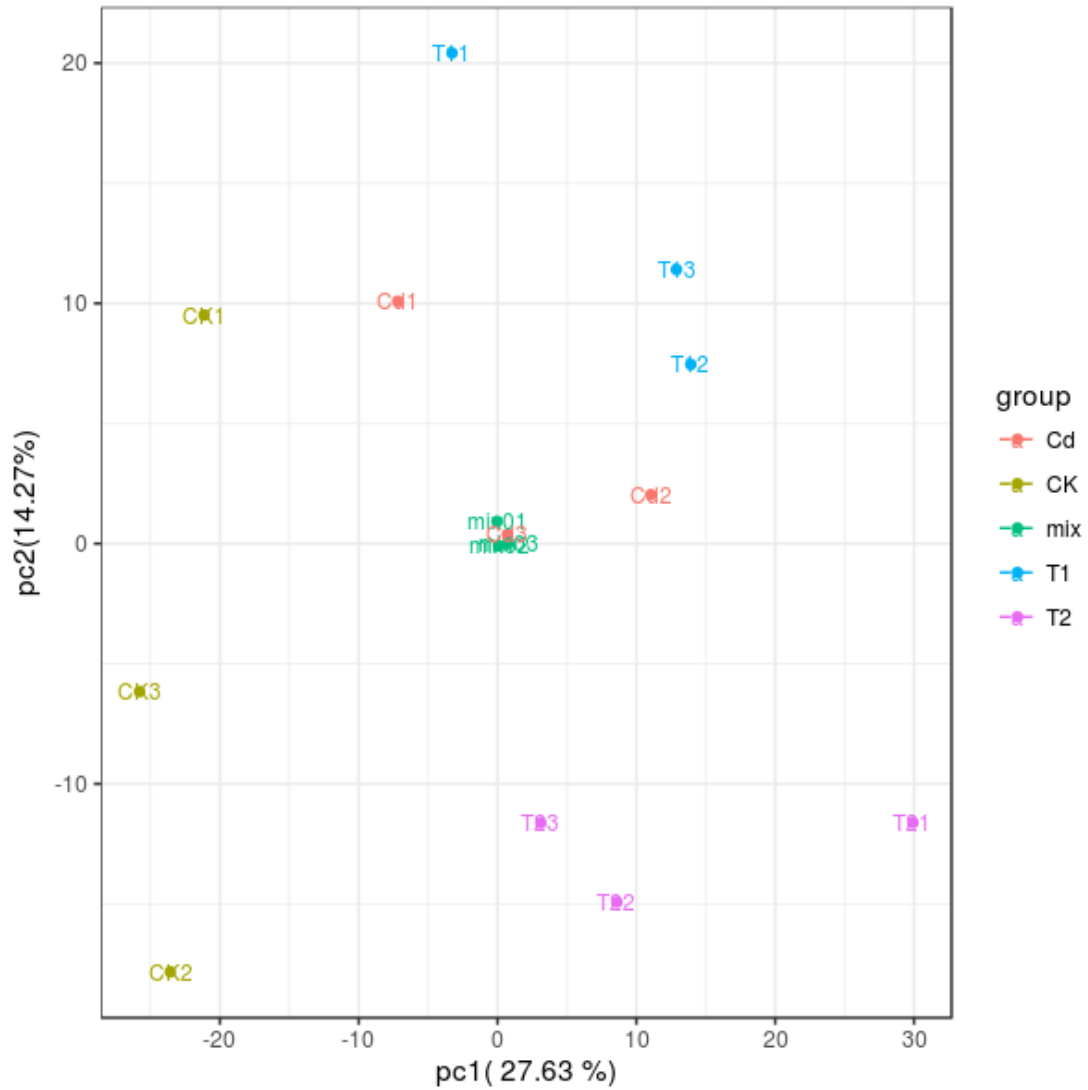


Fig. S4 Principal component analysis (PCA) of the metabolite profiles in tall fescue roots. The analysis was performed on all the metabolites detected in tall fescue roots under different conditions. There were four treatment regimes in this study, including the control (CK), Cd treatment, T1 treatment, and T2 treatment, and each regime had three replicates. They respectively presented the tall fescue seedlings were cultivated in 1/2 Hoagland solution (control, CK), 1/2 Hoagland solution with 50 mg/L Cd²⁺ (CdCl₂•2.5H₂O) (Cd treatment), 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM SNP (T1 treatment), and 1/2 Hoagland solution with 50 mg/L Cd²⁺, 200 μM L-NAME and 100 μM c-PTIO (T2 treatment).

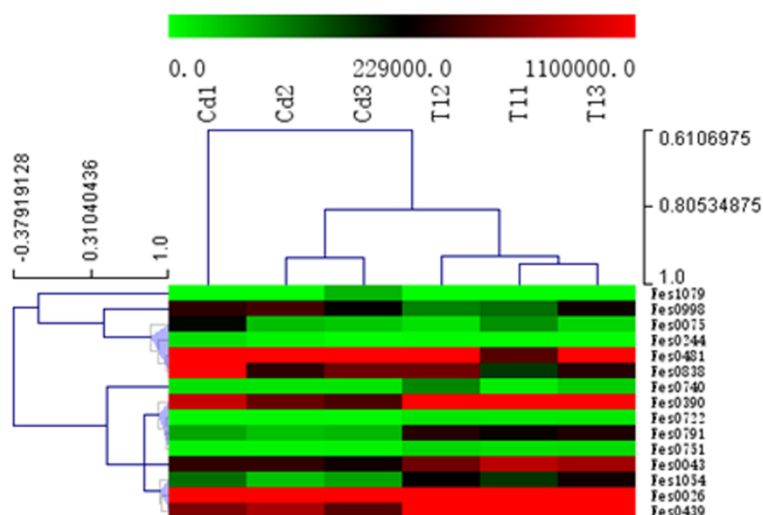


Fig. S5 The hierarchical cluster analysis (HCA) of the differentially expressed metabolites selected from the integrated analysis between the T1 treatment and Cd treatment in tall fescue roots. The tall fescue seedlings were cultivated in 1/2 Hoagland solution with 50 mg/L Cd²⁺ (CdCl₂•2.5H₂O) (Cd treatment) and 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM SNP (T1 treatment), respectively. Each value is the mean of three replicates.

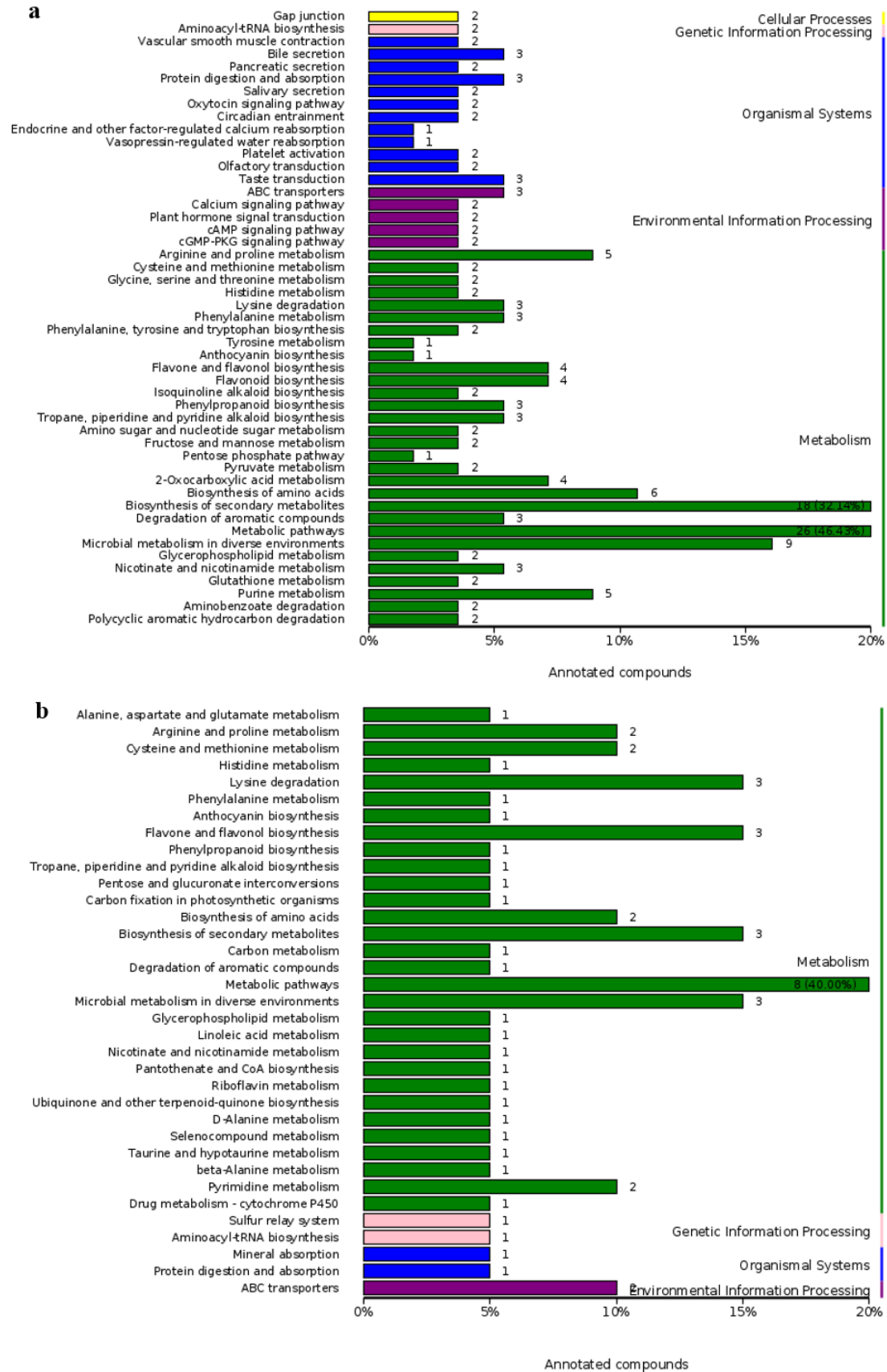


Fig. S6 The distribution of metabolites in different KEGG pathways. (A) the

metabolites in Cd vs Con. (B) the metabolites in T1 vs Cd. There were three regime, comprising Cd, T1 and T2. They respectively presented the tall fescue seedlings were cultivated in 1/2 Hoagland solution with 50 mg/L Cd²⁺ (CdCl₂•2.5H₂O) (Cd treatment), 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM SNP (T1 treatment) and 1/2 Hoagland solution with 50 mg/L Cd²⁺ and 200 μM NG-nitro-L-Arg-methyl ester (L-NAME) and 100 μM 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) (T2 treatment).

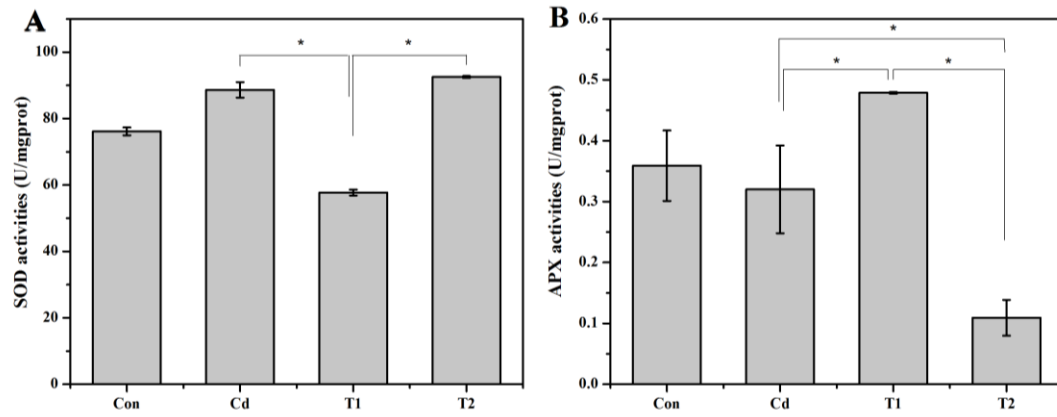


Fig. S7 The activities of SOD (superoxide dismutase) and APX (Ascorbate peroxidase) in tall fescue roots. Values were given as means \pm SD (n = 4). Data about Cd, T1 and T2 treatment were analyzed using one-way Analysis of Variance, followed by LSD test. Asterisks (*) indicate the significant difference at $P < 0.05$.

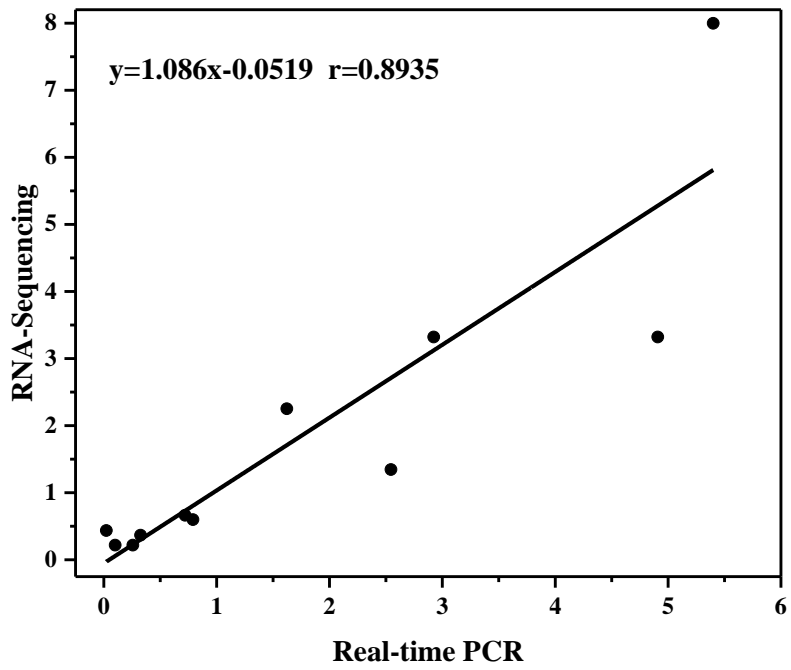


Fig. S8 Correlations of expression level analyzed by RNA-Seq platform (y axis) with data resulted from qRT-PCR (x axis).