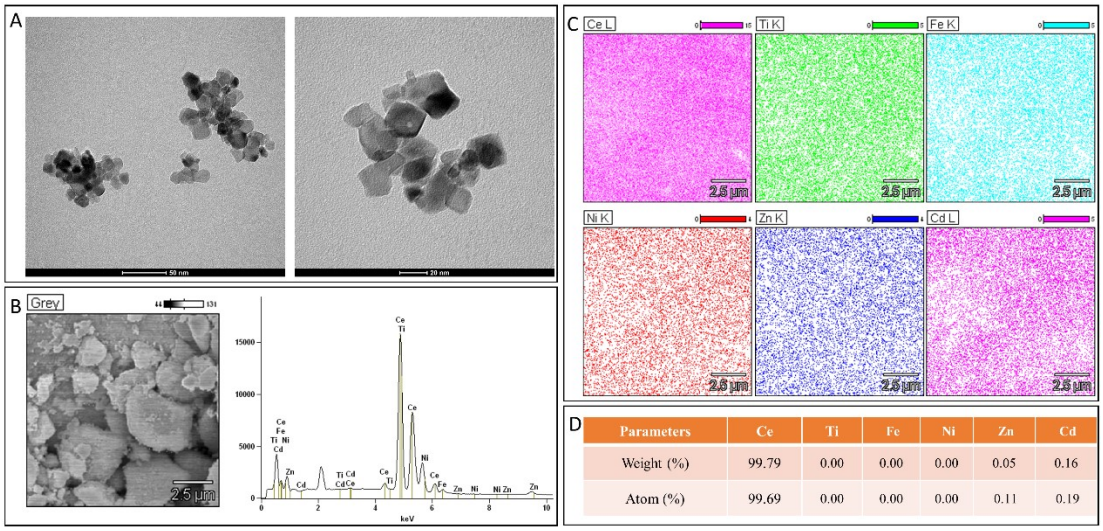
Electronic Supplementary Material (ESI) for Environmental Science: Nano.



Parameters	Ce	Ti	Fe	Ni	Zn	Cd
Weight (%)	99.79	0.00	0.00	0.00	0.05	0.16
Atom (%)	99.69	0.00	0.00	0.00	0.11	0.19

Fig. S1 The characterizations of CeO_2 NPs: (A) SEM images of CeO_2 NPs, (B) EDS spectrum of CeO_2 NPs, (C) EDS elemental mapping images of CeO_2 NPs, and (D) weight and atomic concentration of CeO_2 NPs.

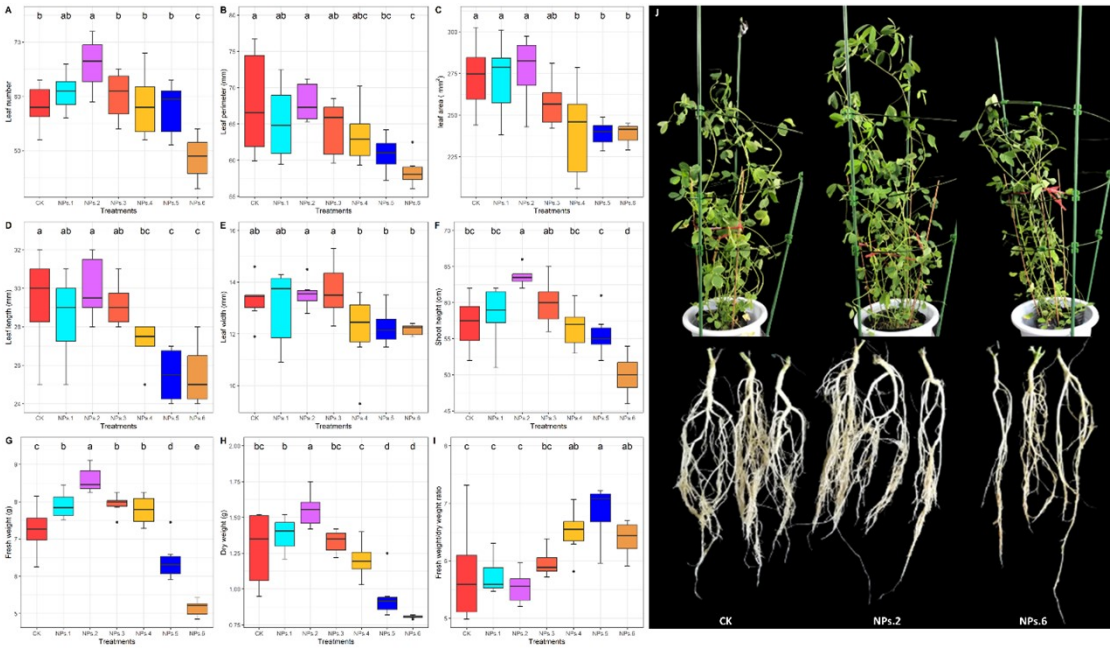


Fig. S2 Effects of CeO_2 NPs on growth traits of alfalfa: (A) leaf number, (B) leaf perimeter, (C) leaf area, (D) leaf length, (E) leaf width, (F) shoot height, (G) fresh weight, (H) dry weight, (I) fresh weight: dry weight ratio, and (J) phenotype of shoots and roots. Data present the means ± SD (n=6). Different letters above the error bars indicate a significant difference at $P < 0.05$.

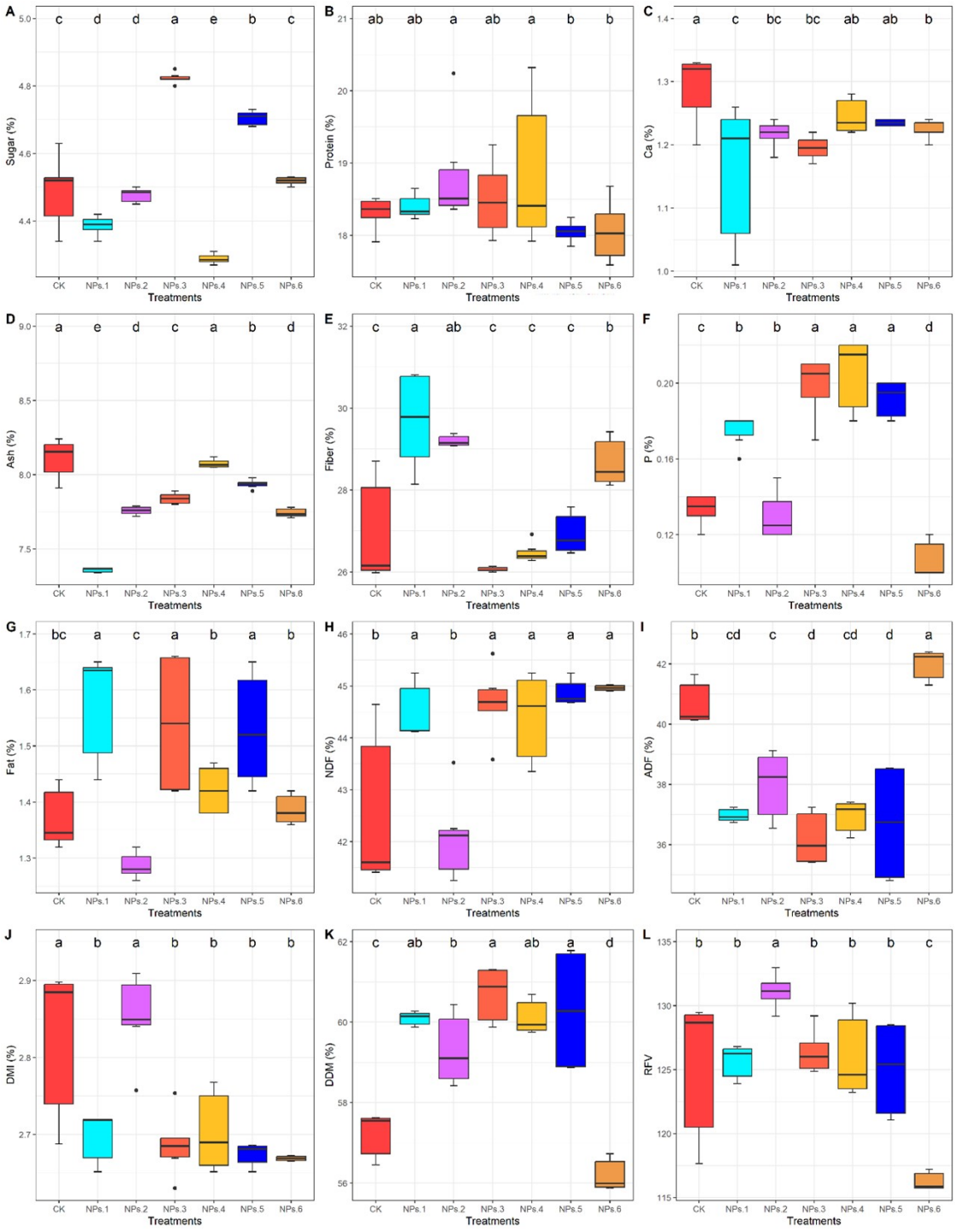


Fig. S3 Effects of CeO_2 NPs on nutritional traits of alfalfa: (A) sugar, (B) protein, (C) Ca, (D) ash, (E) fiber, (F) P, (G) fat, (H) NDF, (I) ADF, (J)DMI, (K) DDM, and (L) RFV. Data present the means ± SD (n=6). Different letters above the error bars indicate a significant difference at $P < 0.05$.

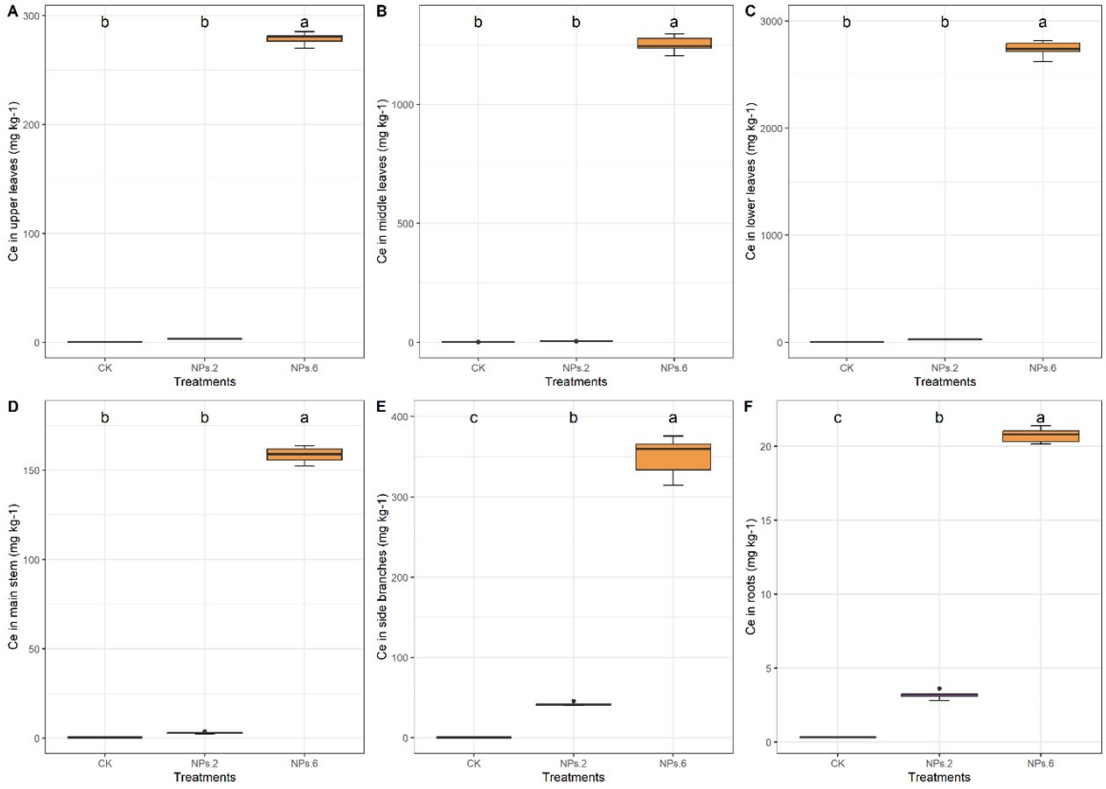


Fig. S4 Effects of CeO_2 NPs on Ce concentration of alfalfa: (A) Ce in upper leaves, (B) Ce in middle leaves, (C) Ce in lower leaves, (D) Ce in main stem, (E) Ce in side branches, and (F) Ce in roots. Data present the means ± SD (n=6). Different letters above the error bars indicate a significant difference at $P < 0.05$.

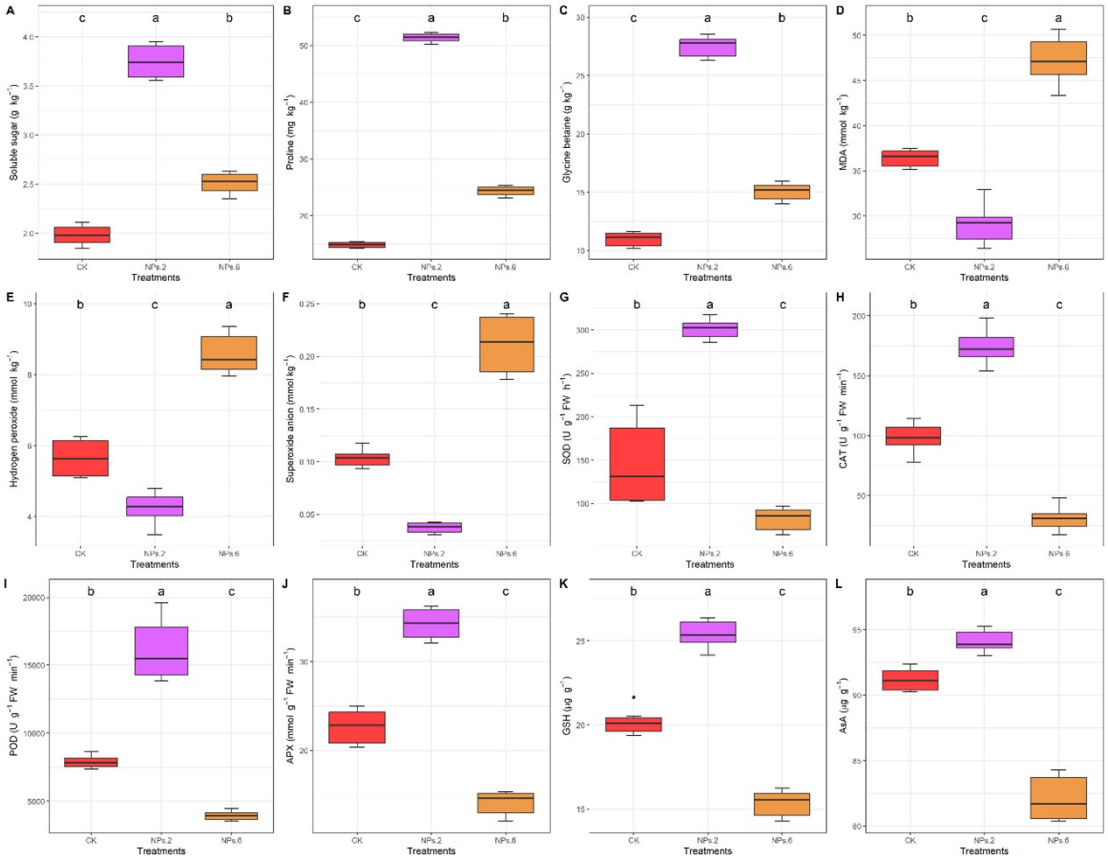


Fig. S5 Effects of CeO_2 NPs on osmotic and redox homeostasis of alfalfa: (A) soluble sugar, (B) proline, (C) glycine betaine, (D) MDA, (E) H_2O_2, (F) $O_2^{\cdot -}$, (G) SOD, (H) CAT, (I) POD, (J) APX, (K) GSH, and (L) AsA. Data present the means ± SD (n=6). Different letters above the error bars indicate a significant difference at $P < 0.05$.

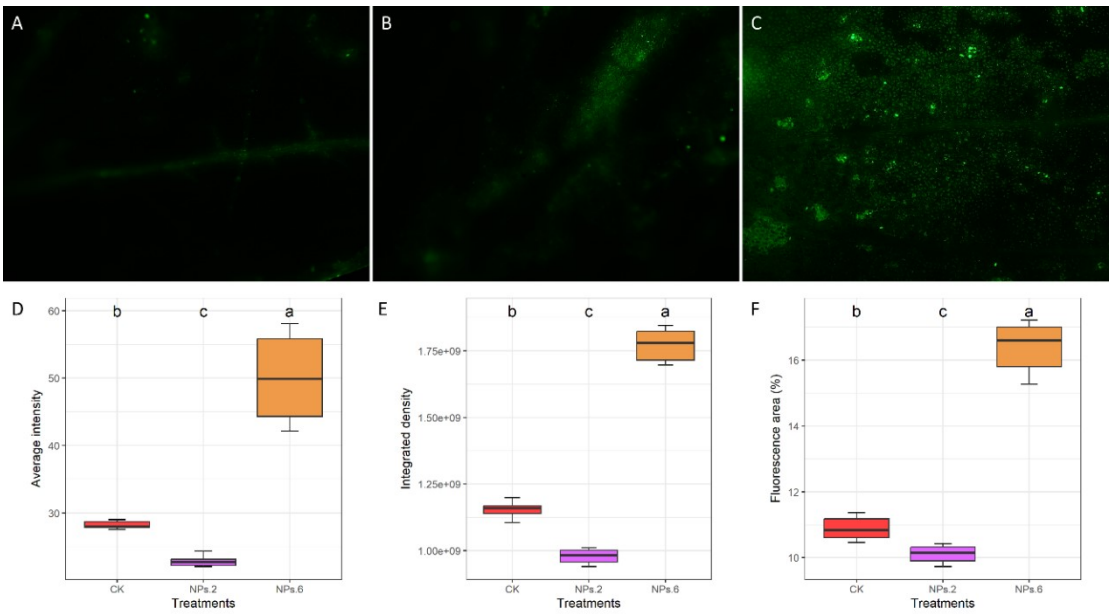


Fig. S6 Effects of CeO_2 NPs on leaf ROS fluorescence intensity of alfalfa: (A)-(C) leaf ROS fluorescence images captured by a 4-fold (200 μm) upright fluorescence microscope for CK, NPs.2, and NPs.6, respectively; (D) -(F) average fluorescence intensity, integrated fluorescence density, and fluorescence area, respectively. Data present the means ± SD (n=6). Different letters above the error bars indicate a significant difference at $P < 0.05$.

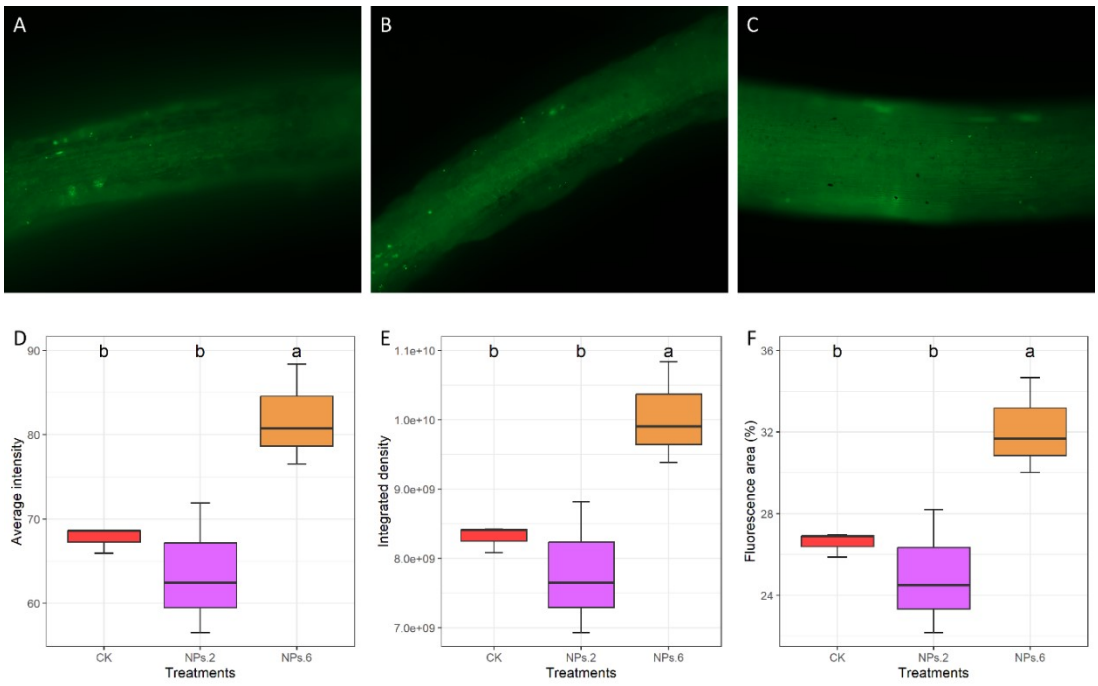


Fig. S7 Effects of CeO_2 NPs on root ROS fluorescence intensity of alfalfa: (A)-(C), fluorescent images of CK, NPs. 2, and NPs.6, respectively; (D)-(F) fluorescence average intensity, fluorescence integrated density, and fluorescence area, respectively. Data present the means ± SD (n=6). Different letters above the error bars indicate a significant difference at $P < 0.05$.

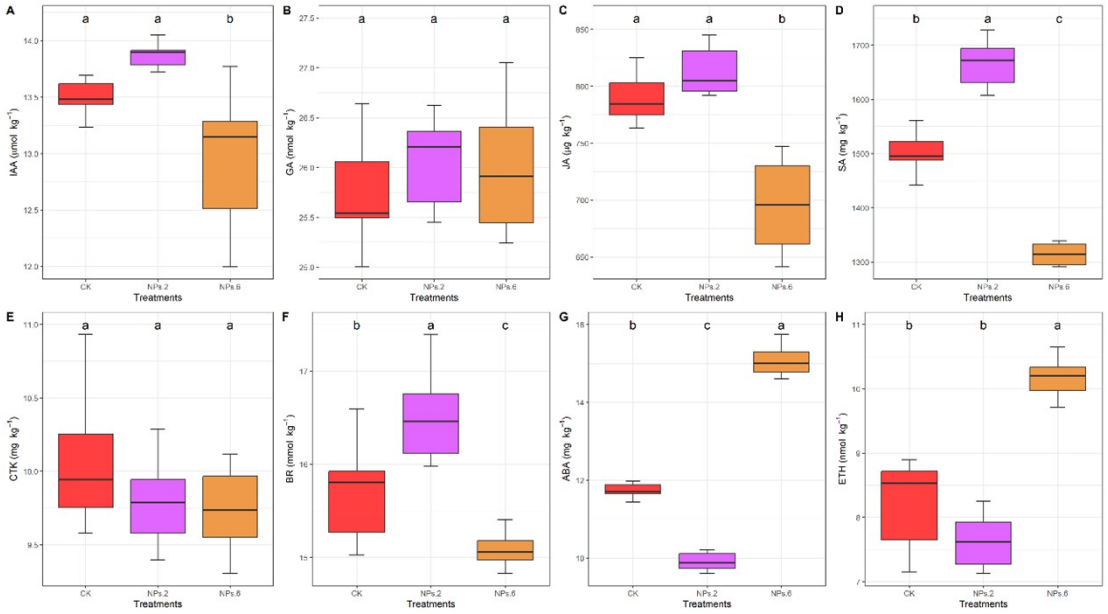


Fig. S8 Effects of CeO_2 NPs on hormones of alfalfa: (A) IAA, (B) GA, (C) JA, (D) SA, (E) CTK, (F) BR, (G) ABA, and (H) ETH. Data present the means ± SD (n=6). Different letters above the error bars indicate a significant difference at $P < 0.05$.

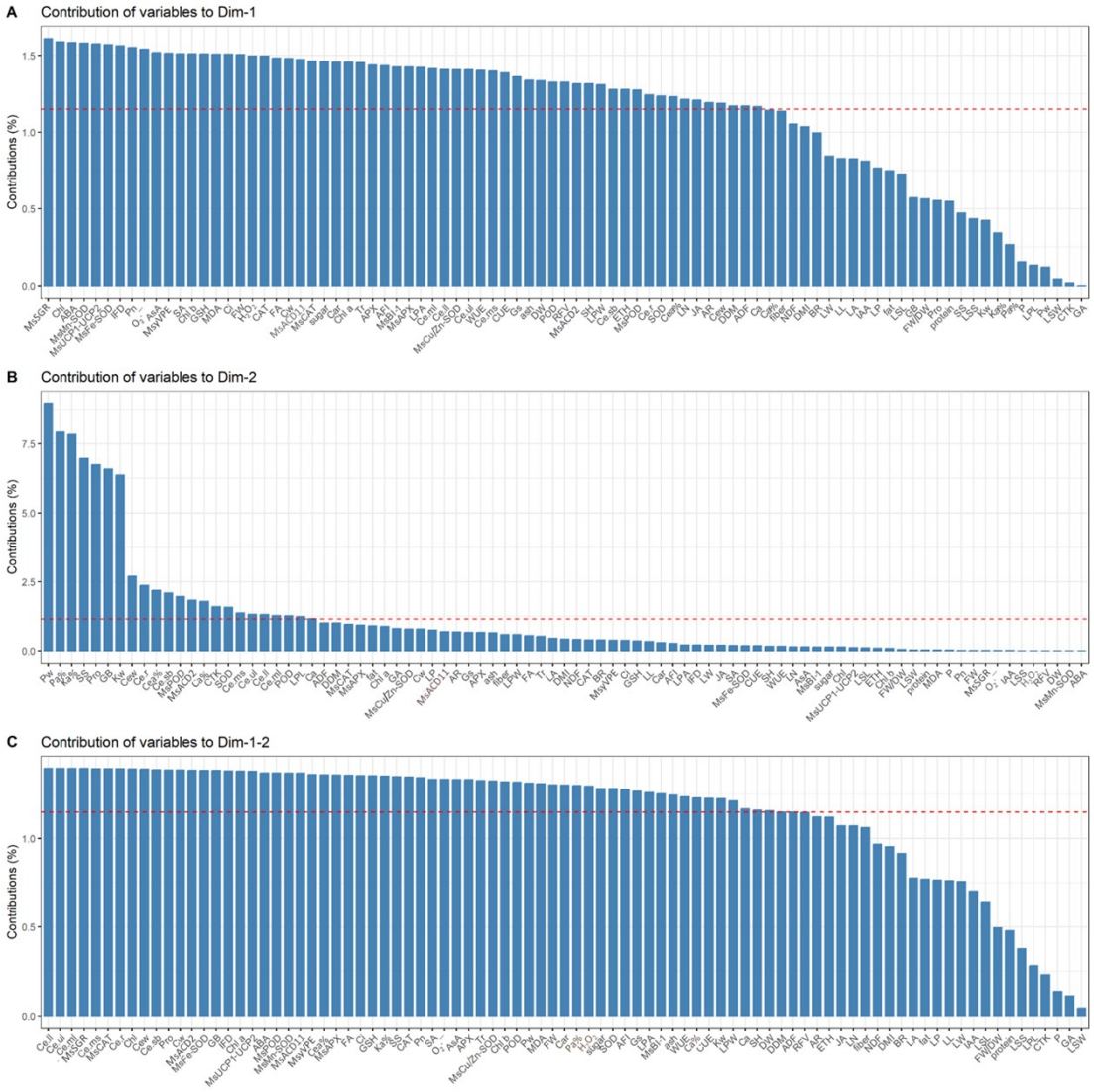


Fig. S9 Contribution of variables to Dim 1 (A), 2 (B) and 1-2 (C).

Table 1 Sequences of the primers used for the genes.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Cu/Zn -SOD	CTCGACAGGACCACATTTCA	AAGGCTCTCCCAATGACTGA
Fe-SOD	GAGTACCATTGGGGAAAGCA	CCATACCTGTGCTGCATTGT
Mn-SOD	GTTGCCAAAGCTGATTCCTC	ACCACCTCCTTCACGAACAG
CAT	CCAAGTCCCACATTCAGGAG	ACTGCTTTCCCAGCCTTGTT
POD	CTACCTGGCCCTCATTTCAA	CTTCTTGGGCTGAATCCGTA
APX	GAAATGCGCTCCTCTTATGC	TGTTAGCACCATGAGCAAGC
SGR	CCTGTGGTCTTGAAGGCATTTG	TGAAAATGTACCCAAACCAAAGC
γVPE	TTTCCTCCGATTACCCTCCCAAG	TCCAGTAGCCATTAGAACCAGCAA
ACD2	GCCCTTGTCTCTCCATCAGC	CCAGCATTACCTTAGCAACAGG
ACD11	GAGACAGATTACGTCGCTAAGG	CCTCACACGTTTCCCTTGTA
BI-1	CTTCTGCCATCTTTGGAGGTTCA	CCTTCTCAACTATTTCCTGCGTGT
UCP1	GGTCAAGTCGAGAATGATGGG	GTTCCAAGATCCTAGCCGTCC

-	AGA	A
UCP2		
β-	TTTGAGACTTTCAATGTGCCCG	TAGCATGTGGGAGTGCATAAC
actin	CC	CCT

Table S2 Eigenvalue and percentage of variance for the first five axes (Dim 1-5) derived from principal component analysis (PCA) and correlation between the axes and the variables.

Variable	Dim 1	Dim 2	Dim 3	Dim 4	Dim 5
Eigenvalue	61.7105465	9.5910921	2.9844664	2.5465088	2.0769855
Variance percent	70.9316626	11.0242437	3.4304212	2.9270216	2.3873397
LN	0.86557097	0.119454	0.069922	-0.04983	-0.14239
LA	0.7146539	-0.20878	0.043288	-0.1813	0.38021
LP	0.68772244	-0.26815	0.277769	-0.01281	0.224702
LL	0.71484196	-0.18012	0.013052	0.155995	0.276192
LW	0.72163889	-0.14016	0.093512	0.202113	-0.1108
SH	0.90064234	0.124558	0.055032	0.02486	-0.09324
FW	0.96363963	0.029532	0.043761	0.024707	0.102073
DW	0.90810032	-0.01088	0.227495	0.030359	0.215141
FW/DW	-0.59115753	0.067085	-0.53466	0.019168	-0.39061
sugar	0.94922433	0.113387	0.093245	-0.18437	-0.07233
protein	0.58207403	0.051923	0.363733	-0.01951	-0.10947
Ca	0.84874841	-0.3346	0.087849	-0.1053	-0.08855
ash	0.90827891	-0.25081	0.288102	0.008404	0.077796
fiber	0.83709601	0.237773	-0.28647	0.048621	-0.16103
P	0.31042361	0.038023	-5.3E-05	-0.4965	0.189129
fat	0.68028765	-0.29412	0.18678	-0.40748	0.073732
NDF	-0.80617481	0.198009	-0.4396	-0.14714	-0.11712
ADF	-0.84968708	-0.31149	-0.01211	0.309897	-0.2496
DMI	0.79943475	-0.20056	0.446316	0.156164	0.115698
DDM	0.84968708	0.311491	0.012112	-0.3099	0.249596
RFV	0.90420501	0.011696	0.298231	-0.03294	0.186854
Pn	0.97828885	0.030105	-0.0783	0.013928	0.083253
Gs	0.91652295	0.251967	0.007642	0.064205	-0.12442
Ci	0.96464623	0.190094	-0.01942	0.010265	-0.07871
Tr	0.94718261	0.222109	-0.10297	0.009448	-0.04058
CUE	0.92548895	-0.13265	-0.10925	0.027863	0.183738
WUE	-0.93039634	-0.12429	0.140983	-0.01035	0.166958
Chl a	0.94817368	-0.29176	-0.04675	-0.02949	-0.02051
Chl b	0.96571019	0.091768	0.022347	0.052055	-0.00261
Chl	0.99035719	-0.112	-0.01416	0.00988	-0.01236
Car	0.94830512	-0.16989	-0.12631	-0.08784	-0.01369
AFI	-	0.159345	-0.00132	0.080358	0.142804

	0.94080975				
IFD	-0.98247137	0.141678	0.018405	0.047073	0.055899
FA	-0.95656002	0.228453	-0.00393	0.068395	0.098134
Ce.ul	-0.93164841	0.356226	0.054237	0.007999	0.026103
Ce.ml	-0.93400138	0.349849	0.057702	0.011288	0.019321
Ce.ll	-0.93215926	0.355027	0.053075	0.00451	0.029343
Ce.ms	-0.9287479	0.362636	0.052871	0.003137	0.029313
Ce.sb	-0.88842533	0.448039	0.074773	-0.01152	-0.00389
Ce.r	-0.87563003	0.476474	0.041067	0.013019	0.042231
MDA	-0.9648956	-0.04994	-0.01902	0.012932	0.07947
H_2O_2	-0.96102994	0.015805	0.07601	-0.00949	0.147628
$O_2 \cdot^-$	-0.97494275	-0.02718	0.002023	0.014525	0.003659
SOD	0.87325602	0.388565	-0.16519	0.023463	-0.05856
CAT	0.96064683	0.194988	0.0133	-0.11507	0.090733
POD	0.90431836	0.348924	0.050199	0.074348	-0.05112
APX	0.94190372	0.251611	-0.10888	0.048455	-0.05899
GSH	0.96526226	0.183869	-0.01958	0.061691	-0.02229
AsA	0.96782112	-0.11859	-0.0514	0.08709	0.033988
IAA	0.70793008	0.025507	-0.35994	0.005565	0.288139
GA	0.03790465	0.278658	0.649776	-0.21596	-0.46252
JA	0.86325539	-0.13773	0.139764	-0.13254	-0.12998
SA	0.96576144	0.136666	-0.03202	-0.11705	-0.01101
CTK	0.10954684	-0.39149	-0.55173	0.315331	-0.11312
BR	0.78371989	0.193733	-0.21767	-0.31159	0.093677
ABA	-0.98856676	-0.00127	0.067109	0.007647	0.027915
ETH	-0.8882954	0.097703	0.202943	-0.06964	0.273621
SS	0.54077038	0.817968	-0.10819	0.029642	0.095543
Pro	0.58461002	0.804466	-0.03505	0.034423	0.063845
GB	0.59464463	0.79526	-0.07407	0.030696	0.058618
AR	-0.85741995	0.255464	0.240266	-0.05591	-0.14929
Cw	0.95562176	-0.27485	-0.0689	-0.02295	-0.03465
Pw	0.27393044	-0.92751	0.024988	-0.02612	-0.03981
Kw	0.51236073	-0.78139	-0.15269	0.08452	-0.04925

Cew	-0.85634772	0.508636	0.05323	0.02263	0.040579
Ca%	0.83968421	0.413825	-0.09574	-0.11319	0.094984
Pa%	0.40686231	-0.87194	0.036178	0.052035	-0.17053
Ka%	0.46096879	-0.86724	-0.03538	-0.0042	-0.03375
Cea%	-0.87152865	0.458044	0.035442	0.032382	0.071764
MsCu/Zn-SOD	0.93167915	0.274947	0.012046	0.140202	-0.02984
MsFe-SOD	0.98448695	0.133076	-0.04968	-0.0007	0.019226
MsMn-SOD	0.98767474	0.007407	-0.05374	0.021254	0.013416
MsPOD	0.88738901	0.434857	-0.01346	0.044172	-0.02984
MsCAT	0.94981661	0.302889	-0.03302	0.027817	0.019756
MsAPX	0.93791868	0.29867	-0.07178	-0.00405	0.031427
MsACD2	0.90111118	0.419158	-0.01152	0.091307	-0.0198
MsACD11	0.95367026	0.256745	0.009597	0.093406	-0.06044
MsSGR	-0.99691269	-0.02846	0.052826	0.014084	-0.00768
MsγVPE	-0.9663078	-0.19149	0.083254	-0.00705	-0.06595
MsBI-1	0.93805137	0.115089	0.018533	0.198228	-0.10971
MsUCP1-UCP2	0.98611534	-0.10439	-0.01743	0.026991	-0.07261
LPL	0.28695307	0.344991	0.321026	0.523938	-0.40913
LPW	0.89912846	0.236789	-0.06737	0.150541	-0.22761
LPA	0.93681477	0.142799	-0.21782	-0.09378	-0.1214
LSL	0.67008438	0.099405	0.493089	-0.0361	-0.23412
LSW	0.16611457	-0.05603	-0.03825	0.766083	0.463174
LSS	0.5189811	0.016042	0.260301	0.627829	0.241363