Supplementary material

Selenium Nanobiostimulants Attenuate Copper-Induced Oxidative Damage in *Brassica napus* Through Genotype-Specific Antioxidant and Metabolic Adaptation

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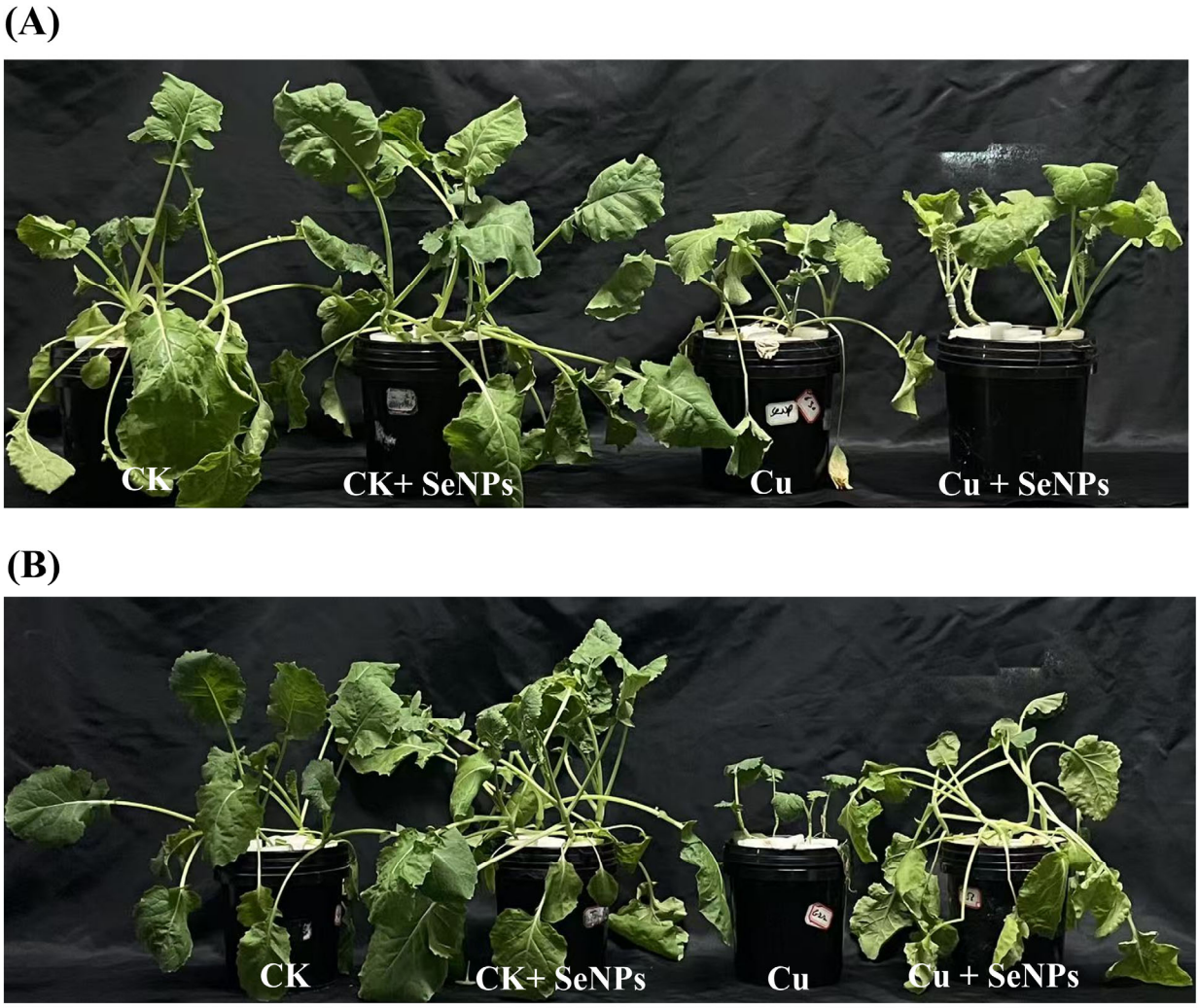


Figure S1. Phenotypic responses of *B. napus*: (A) Zheda 635, the tolerant genotype; (B) Zheda 622, the sensitive genotype under different treatment conditions.

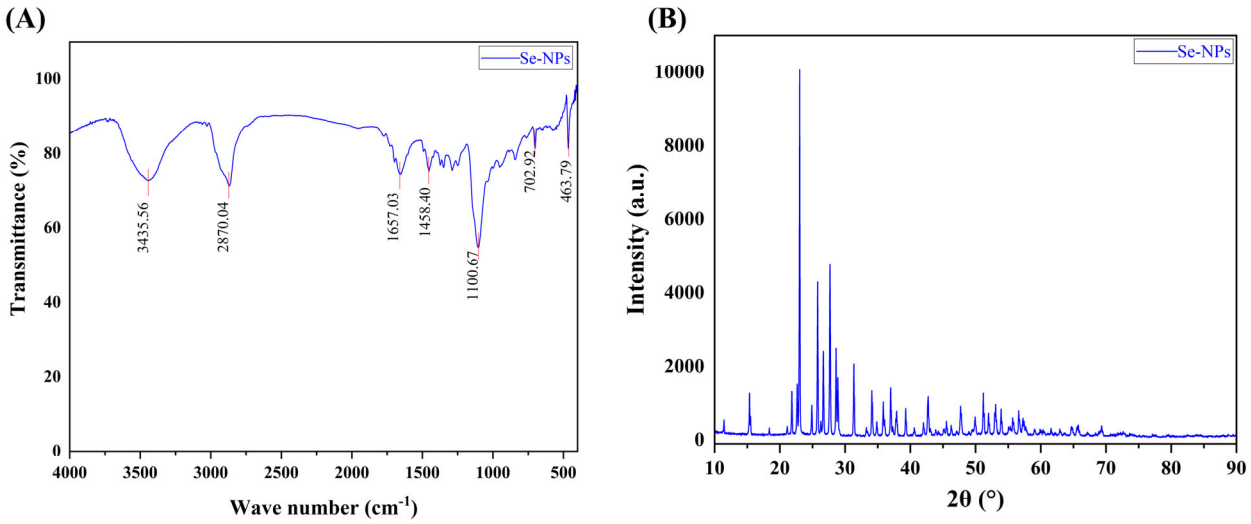


Figure S2. Physicochemical characterization of selenium nanoparticles (SeNPs) showing (A) FTIR spectrum and (B) XRD pattern

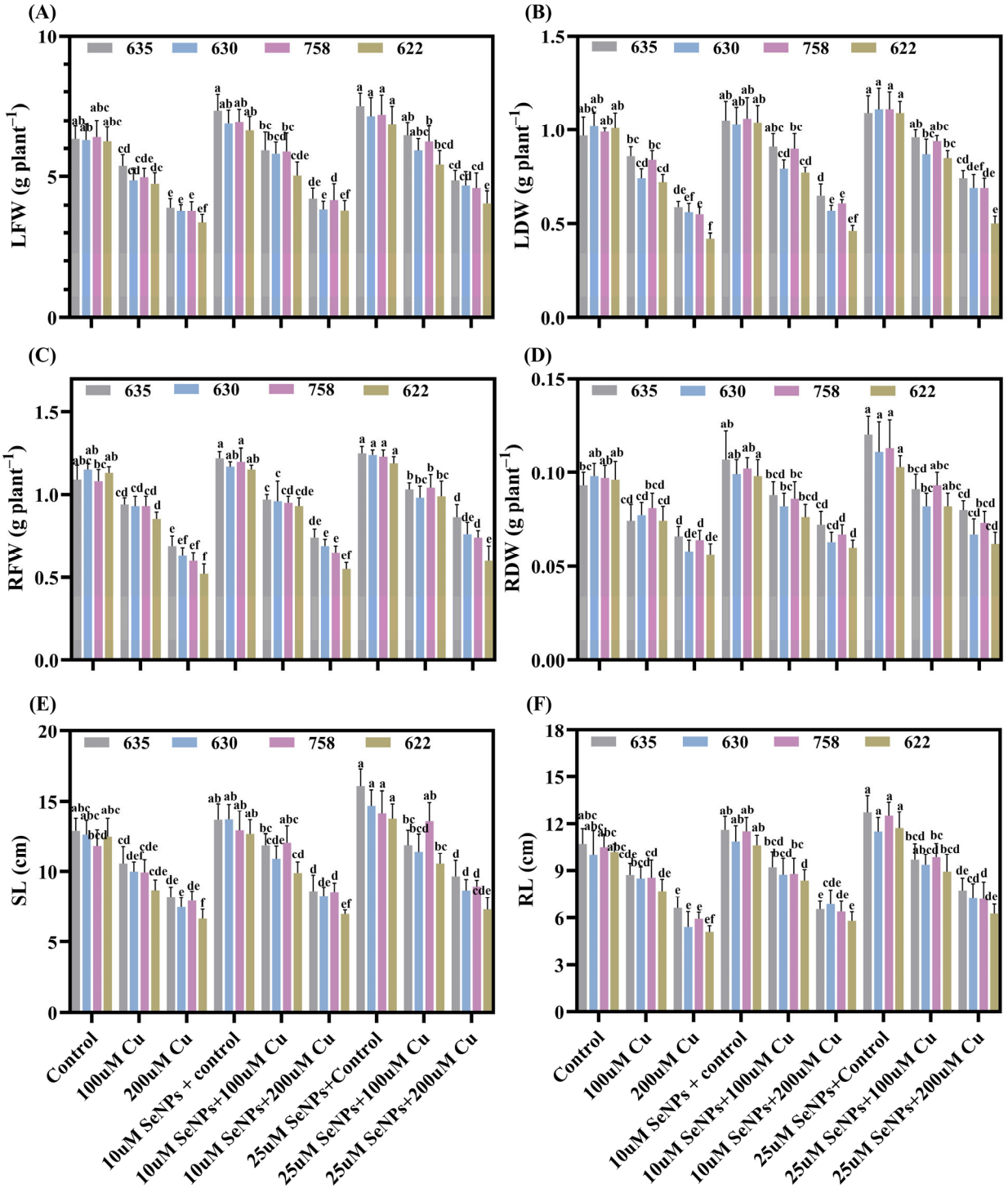


Figure S3. Synergistic effects of copper (0, 100 and 200 µM), and exogenous SeNPs (10 and 25 µM) and their interactions on growth parameters in *B. napus* cultivars (Zheda 635, Zheda 630, ZS 758 and Zheda 622): (A) leaf fresh weight (LFW), (B) leaf dry weight (LDW), (C) root fresh weight (RFW), (D) root dry weight (RDW), (E) shoot length (SL) and (F) root length (RL). Bars are presented as mean ± SD (n = 3). Bars sharing the same lowercase letter are not significantly different, whereas bars with different letters differ significantly according to Tukey's HSD test at $p \leq 0.05$, with comparisons made within each cultivar.

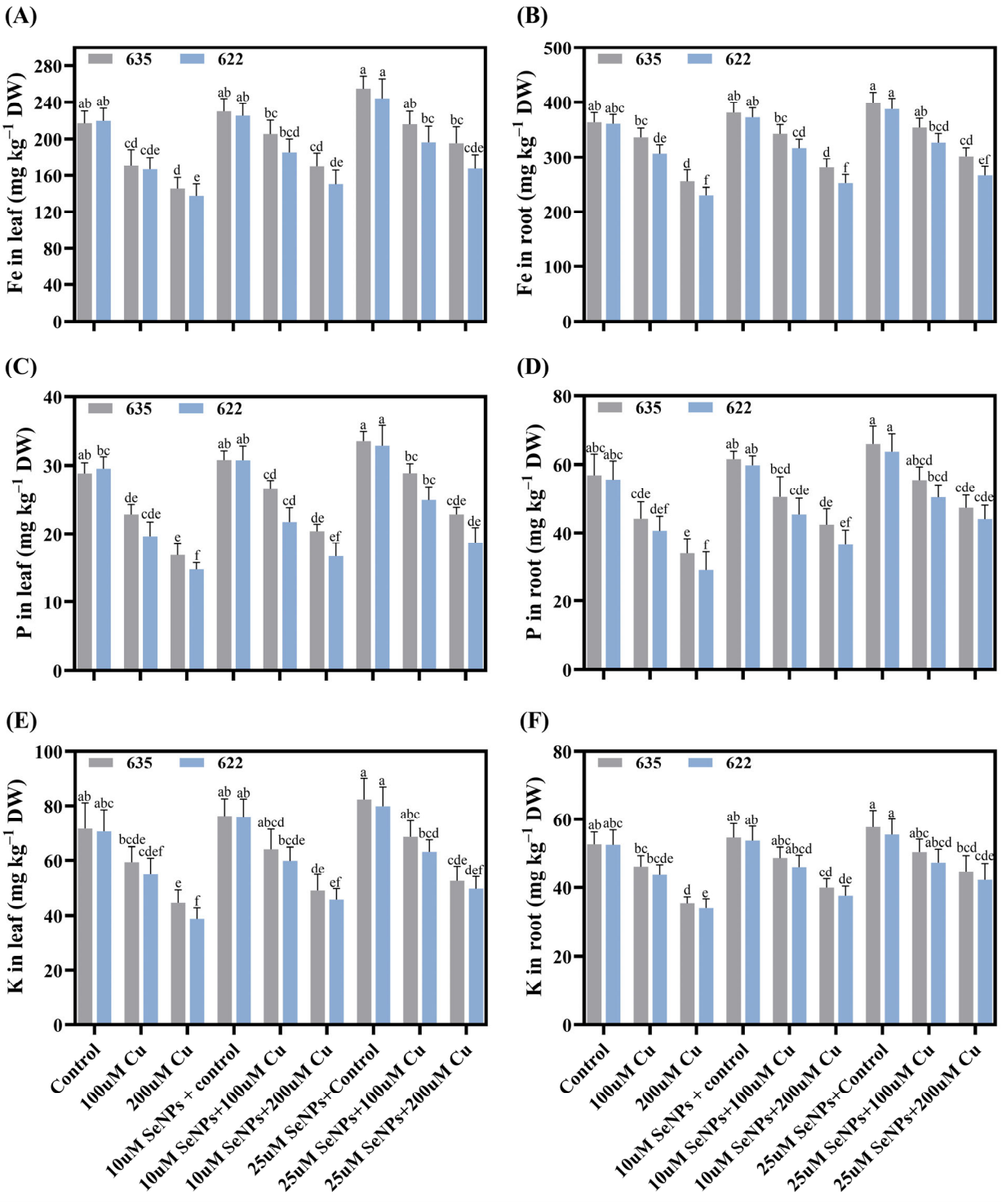


Figure S4. Synergistic effects of copper (0, 100 and 200 μM) and exogenous SeNPs (10 and 25 μM) and their interactions on nutrient contents in leaf and root of *B. napus* cultivars in Zheda 635 (most tolerant) and Zheda 622 (most sensitive): (A) Fe in leaf, (B) Fe in root, (C) P in leaf, (D) P in root, (E) K in leaf, and (F) K in root. Bars are presented as mean ± SD (n = 3). Bars sharing the same lowercase letter are not significantly different, whereas bars with different letters differ significantly according to Tukey's HSD test at $p \leq 0.05$, with comparisons made within each cultivar.

Table S1. Treatments are shown in the completely randomized design.

Treatment No.	Description
T0	Control (CK)
T1	100 μM Cu (alone)
T2	200 μM Cu (alone)
T3	10μM SeNPs + control
T4	10μM SeNPs + 100μM Cu
T5	10μM SeNPs + 200μM Cu
T6	25μM SeNPs + Control
T7	25μM SeNPs + 100μM Cu
T8	25μM SeNPs + 200μM Cu

Table S2. Oligonucleotide primer sequences used for gene expression (qRT-PCR) of antioxidants analysis

Gene name	Forward	Reverse
BnaSOD	ACGGTGTGACCACTGTGACT	GCACCGTGTTGTTTACCATC
BnaCAT	TCGCCATGCTGAGAAGTATC	TCTCCAGGCTCCTTGAAGTT
BnaAPX	ATGAGGTTTGACGGTGAGC	CAGCATGGGAGATGGTAGG
BnaGR	AAGCTGGAGCTGTGAAGGTT	AGACAGTGTTCGCAAAGCAG
Actin	TTGGGATGGACCAGAAGG	TCAGGAGCAATACGGAGC

Table S3. Eigenvalues, proportion of variance, and cumulative variance explained by the first six principal components derived from 18 physiological traits measured across four *Brassica napus* cultivars (622, 630, 635, 758) under nine SeNPs and Cu stress treatments (n = 36). Data were centered and scaled prior to PCA.

PC	Eigenvalue	Variance (%)	Cumulative (%)	Interpretation
PC1	10.875	58.7	58.7	Growth vs. stress axis
PC2	6.201	33.5	92.2	ROS and root trait axis
PC3	0.774	4.2	96.4	Minor residual variation
PC4	0.357	1.9	98.3	Minor residual variation
PC5	0.146	0.8	99.1	Negligible
PC6	0.057	0.3	99.4	Negligible

Table S4. Eigenvector loadings, percentage contributions, and squared cosine values (cos^2) for each physiological trait on PC1 and PC2. Traits are sorted by PC1 loading (descending). Contributions were calculated as ($loading^2$ / $\Sigma loadings^2$) × 100.

Trait	PC1 Loading	PC1 Contrib. (%)	PC2 Loading	PC2 Contrib. (%)	Cos^2 (PC1)	Cos^2 (PC2)
LDW	+0.3009	9.05	-0.0144	0.02	0.091	0.000
RL	+0.3006	9.04	-0.0045	0.00	0.090	0.000
SL	+0.3003	9.02	-0.0004	0.00	0.090	0.000
LFW	+0.3001	9.00	-0.0145	0.02	0.090	0.000
Pn	+0.2995	8.97	-0.0547	0.30	0.090	0.003
Gs	+0.2287	5.23	-0.2592	6.72	0.052	0.067
Chl b	+0.2171	4.71	-0.2702	7.30	0.047	0.073
H_2O_2 root	-0.0240	0.06	+0.4034	16.27	0.001	0.163
H_2O_2 leaf	-0.0299	0.09	+0.4018	16.15	0.001	0.161
RFW	-0.1508	2.28	-0.3178	10.10	0.023	0.101
RDW	-0.1621	2.63	-0.3032	9.20	0.026	0.092
Chl a	-0.1969	3.88	-0.2838	8.05	0.039	0.081
MDA root	-0.2068	4.27	+0.2938	8.63	0.043	0.086
Tr	-0.2160	4.66	-0.2556	6.53	0.047	0.065
Fv/Fm	-0.2282	5.21	-0.2393	5.73	0.052	0.057
MDA leaf	-0.2401	5.77	+0.2041	4.16	0.058	0.042
$O_2^{\bullet-}$ root	-0.2789	7.78	-0.0831	0.69	0.078	0.007
$O_2^{\bullet-}$ leaf	-0.2890	8.35	+0.0347	0.12	0.084	0.001