



**Appendix A Transmission electron microscopic (TEM) image and size distribution of the CuO NPs.** It can be seen that CuO NPs are kind of agglomerate and the particle sizes ranged from 10-100 nm. Some bigger size nanoparticles were observed duo to the agglomeration.

**Appendix B The selected genes and the primes used for gene expression**

GenBank accession no.	Genes	Gene products	Functions	Primers
AF212184.1	<i>hrs203J</i>	Cell death associated protein	Participates in response and cell death	F: CACCCGAAGTCAAATTCATGG; R: GTTCAGGTAAGTAGATGCGGAG
X67076.1	<i>NmIMSP</i>	Inhibitor of microbial serine proteinases	Inhibits microbial serine proteinases	F: TTCACTCTTTCAACCCCTCAC; R: TTTCTTTGTCACTCCTGGG
D90197.1	<i>PR1b</i>	Pathogenesis-related proteins-1b	Contributes to systemic acquired resistance in plants	F: ACTGCAACCTCGTACATTCTC; R: TTTCTCATCGACCCACATCTC
D64052.1	<i>P450-1</i>	cytochrome P450-1 monooxygenase	Catalyze oxidative reactions in plant secondary metabolism	F: TCAGAGCCAATCCTTTTCCC; R: AACGCATCAGGTCTCCAAG
AF368381.1	<i>P450-2</i>	cytochrome monooxygenase P450-2	Catalyze oxidative reactions in plant secondary metabolism	F: CAGCACTATGAGTTGATCCCG; R: CCAAAGGCTCGTTAGTCGTAG
L18908.1	<i>L25</i>	Ribosomal protein		F:CCACCGACCTAAGACTTTGAAG; R: TCTTCATTGCAGACTCTGTGG

### Appendix C Effects of CuO NPs on tobacco seed germination

Treatment (mg/L)	Mean germination time (d)	Germination Rate (%)	Germination potential
6.25	3.46±0.05 aA	99.33±1.08 aA	38.12±5.73 aA
12.5	3.40±0.17aA	99.33±1.15aA	39.67±6.51aA
25	2.84±0.14bB	98.00±2.65aA	70.33±7.64bB
50	2.55±0.06cC	98.33±0.58aA	79.33±4.73bBcC
100	2.47±0.13cC	99.67±0.58aA	88.67±3.51cCd
200	2.41±0.04cC	99.33±1.15aA	91.67±5.13dC
Control	3.48±0.05aA	99.00±1.00aA	32.67±3.06aA

Values were averaged from three independent replicates, with significant differences marked with lowercase letters ( $P<0.05$ ).

### Appendix D Effects of CuO NPs on growth traits of tobacco seedlings

Treatment (mg/L) <sup>1)</sup>	Plant height (cm)	Stem Diameter (cm)	Leaf length (cm)	Leaf width (cm)	Dry weight (g)
12.5	15.80±1.85a	0.41±0.03a	22.40±1.08a	12.40±0.61a	1.38±0.19a
25	14.88±1.90a	0.44±0.03ab	21.25±2.05a	11.43±1.22a	1.38±0.42a
50	14.98±2.56a	0.44±0.02ab	21.40±1.23a	11.23±1.13a	1.44±0.39a
100	16.30±1.53a	0.43±0.02ab	24.83±1.52a	11.63±0.66a	1.50±0.19a
200	17.40±2.03a	0.40±0.01a	23.40±1.67a	11.30±1.29a	1.36±0.32a
Control	17.13±2.10a	0.43±0.02ab	22.35±1.32a	11.58±1.08a	1.61±0.31a

<sup>1)</sup>10 mL of different concentrations of CuO NPs were applied to 4-5 leaf stage tobacco seedlings using irrigation method, and the treatment were conducted every 7 days. The tobacco seedlings were kept at a constant temperature of 27°C for 30 d. Sterile water was used as a control.

Values were averaged from three independent replicates, with significant differences marked with lowercase letters ( $P<0.05$ ).