



# Brassinosteroids Mitigate Cadmium Effects in Arabidopsis Root System without Any Cooperation with Nitric Oxide

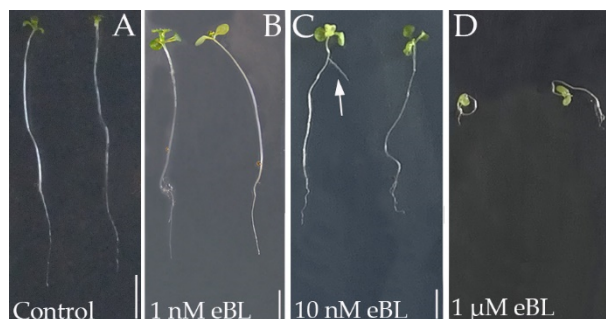
Federica Della Rovere <sup>1</sup>, Diego Piacentini <sup>1</sup>, Laura Fattorini <sup>1</sup>, Nicoletta Girardi <sup>1</sup>, Dario Bellanima <sup>1</sup>,  
Giuseppina Falasca <sup>1</sup>, Maria Maddalena Altamura <sup>1</sup> and Camilla Betti <sup>2,\*</sup>

<sup>1</sup> Department of Environmental Biology, Sapienza University of Rome, 00185 Rome, Italy; federica.dellarovere@uniroma1.it (F.D.R.); diego.piacentini@uniroma1.it (D.P.); laura.fattorini@uniroma1.it (L.F.); nicoletta.girardi@outlook.it (N.G.); dario.bellanima@libero.it (D.B.); giuseppina.falasca@uniroma1.it (G.F.); mariamaddalena.altamura@uniroma1.it (M.M.A.)

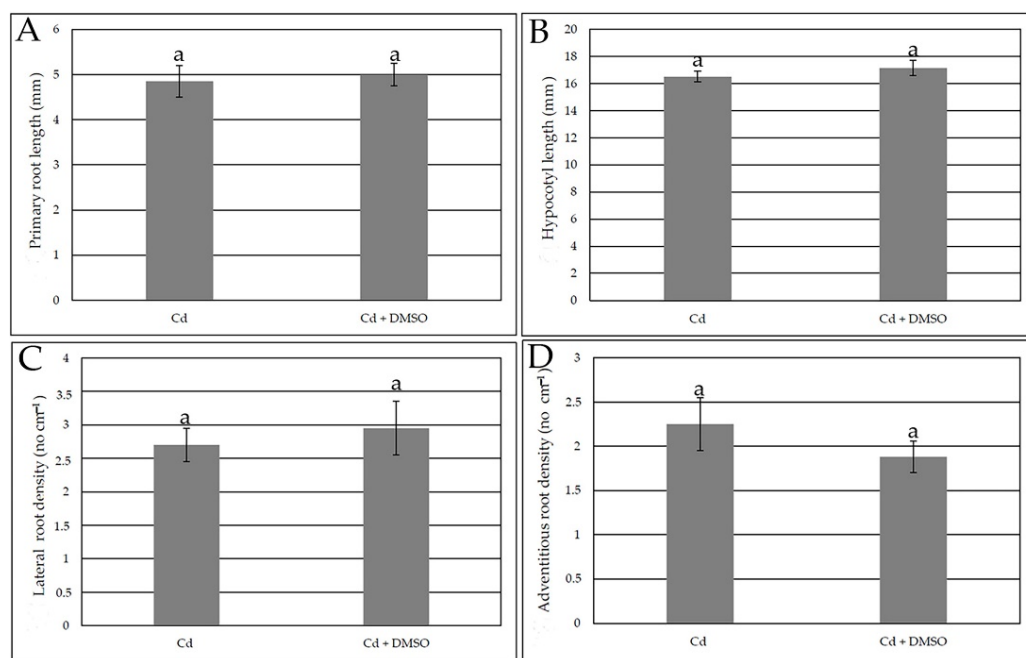
<sup>2</sup> Department of Biosciences, University of Milan, 20133 Milan, Italy

\* Correspondence: camilla.betti@unimi.it

## Supplementary materials

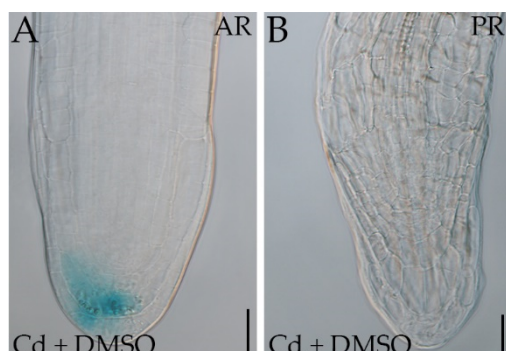


**Figure S1.** Stereomicroscope images of *A. thaliana* seedlings (Col ecotype) cultured for 9 days under continuous darkness followed by 7 days under 16 h light/8 h darkness photoperiod on  $\frac{1}{2}$  MS medium (see Section 4) (Control treatment, **A**), or on the same medium plus either 1 nM eBL (**B**), or 10 nM eBL (**C**), or 1  $\mu$ M eBL (**D**). Arrow shows the presence of an already elongated adventitious root along the hypocotyl. Bars = 5 mm.

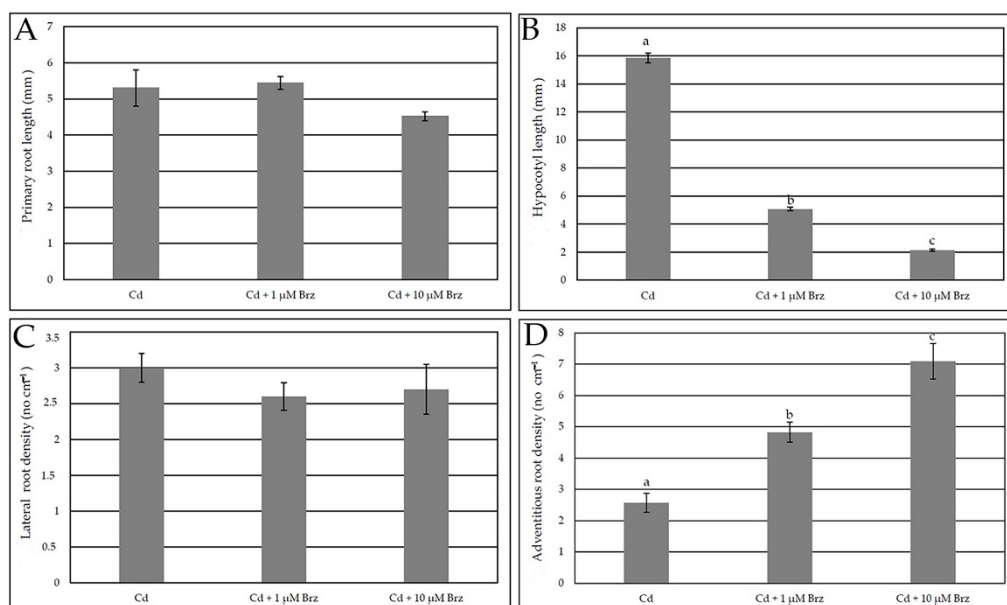


**Figure S2.** Primary root mean length ( $\pm$ SE) (**A**), hypocotyl mean length ( $\pm$ SE) (**B**), mean density of lateral roots ( $\pm$ SE) (**C**) and mean density of adventitious roots ( $\pm$ SE) (**D**) of *Arabidopsis thaliana* Col-0 seedlings cultured for 9 days under continuous darkness followed by 7 days under 16 h light/8 h

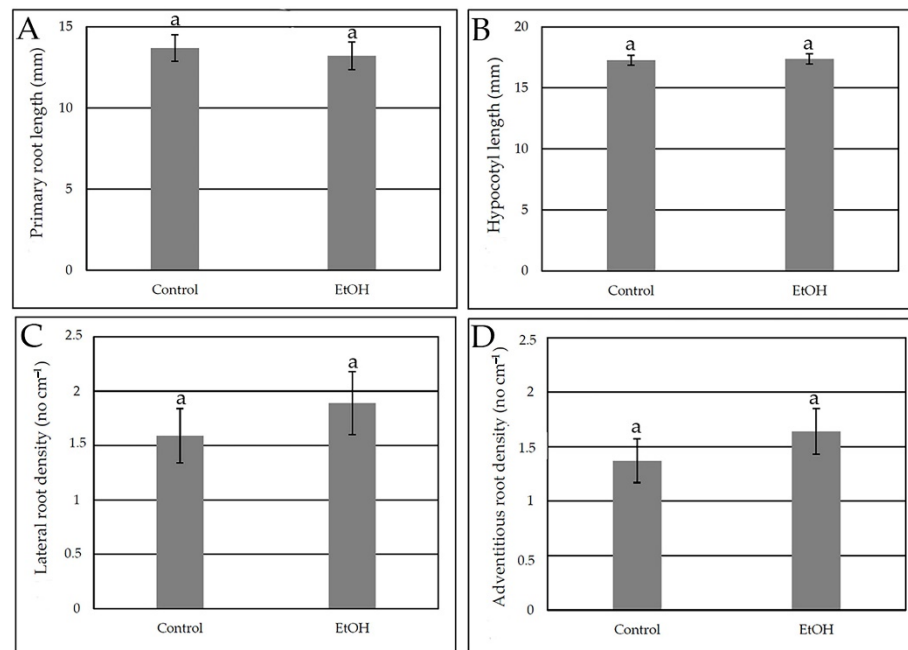
darkness photoperiod on 60  $\mu\text{M}$   $\text{CdSO}_4$  (Cd) or on 60  $\mu\text{M}$   $\text{CdSO}_4$  (Cd) plus DMSO in the same quantity added for obtaining the 10  $\mu\text{M}$  Brz medium. Columns followed by the same letters are not significantly different. N = 30. Data from the third replicate.



**Figure S3.** *DR5::GUS* expression in adventitious roots (AR) (A) and in primary roots (PR) apices (B) of *Col* seedlings cultured for 9 days under continuous darkness followed by 7 days under 16 h light/8 h darkness photoperiod on  $\frac{1}{2}$  MS medium (see Section 4) and treated with 60  $\mu\text{M}$   $\text{CdSO}_4$  (Cd) combined with the DMSO, solvent of Brz. Bars = 20  $\mu\text{m}$ .



**Figure S4.** Primary root mean length ( $\pm\text{SE}$ ) (A), hypocotyl mean length ( $\pm\text{SE}$ ) (B), mean density of lateral roots ( $\pm\text{SE}$ ) (C) and mean density of adventitious roots ( $\pm\text{SE}$ ) (D) of *Arabidopsis thaliana* *Col* seedlings cultured for 9 days under continuous darkness followed by 7 days under 16h light/8 h darkness photoperiod on 60  $\mu\text{M}$   $\text{CdSO}_4$  (Cd) or on 60  $\mu\text{M}$   $\text{CdSO}_4$  (Cd) + 1  $\mu\text{M}$  Brz, or 60  $\mu\text{M}$   $\text{CdSO}_4$  (Cd) + 10  $\mu\text{M}$  Brz. Different letters show significant differences for  $P < 0.0001$ . Columns followed by no letter are not significantly different. N = 30. Data from the third replicate.



**Figure S5.** Primary root mean length ( $\pm$ SE) (A), hypocotyl mean length ( $\pm$ SE) (B), mean density of lateral roots ( $\pm$ SE) (C) and mean density of adventitious roots ( $\pm$ SE) (D) of *Arabidopsis thaliana* Col seedlings cultured for 9 days under continuous darkness followed by 7 days under 16 h light/8 h darkness photoperiod on  $\frac{1}{2}$  MS medium (see Materials and Methods) (Control treatment) or on the same medium plus 0.1% EtOH, i.e. the highest EtOH concentration in the culture media containing eBL. Columns followed by the same letters are not significantly different. N=30. Data from the first replicate.