Phytotoxicity of carbon nanotubes in soybean is associated with disturbances of zinc homeostasis

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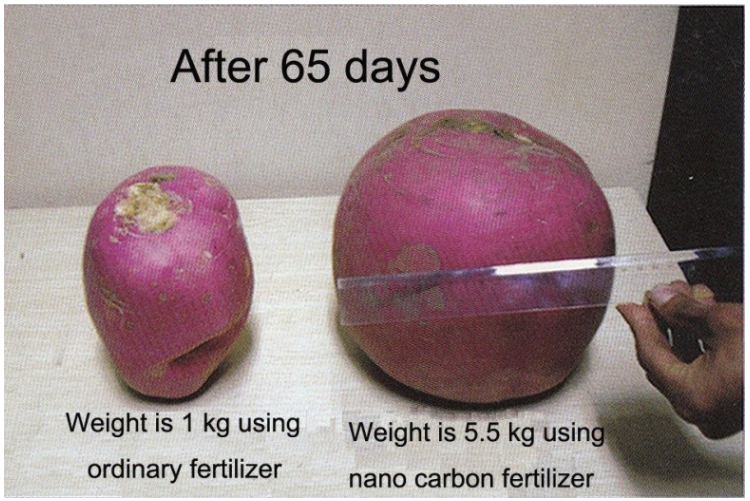
Nanocarbon use in agriculture

Plant Foliar Fertilizer: Nano Carbon Sol

- **Major Contents:** Nano-carbon : ≥5‰ , Particle Diameter: 10～30nm
- **Major Elements:** C, H, O; **Minor and trace elements:** Na, Mg, Al, Si, P, S, Cl, K, Ca
- **Product Features:** Black sol, strong adsorption and conductivity
- **Advantage:** Increase crop yields **(field crops up to 5-15%, vegetables up to 15-30%)**.

[http://www.huanongnano.com/hn/Photo_Show.asp?InfoId=211&ClassId=48]



Nanocarbon Fertilizer

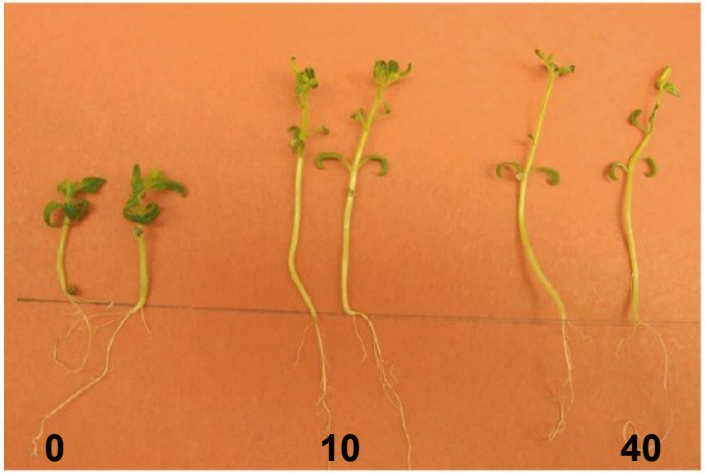
Qinhuangdao Taiji Ring Nano-Products Co., Ltd. (China)

"…Nano-Product Co., ltd has not published research achievements because of the unpredictability of nanotechnology".

[Zheng, Y et al., 2014]

CNT: positive vs negative effects

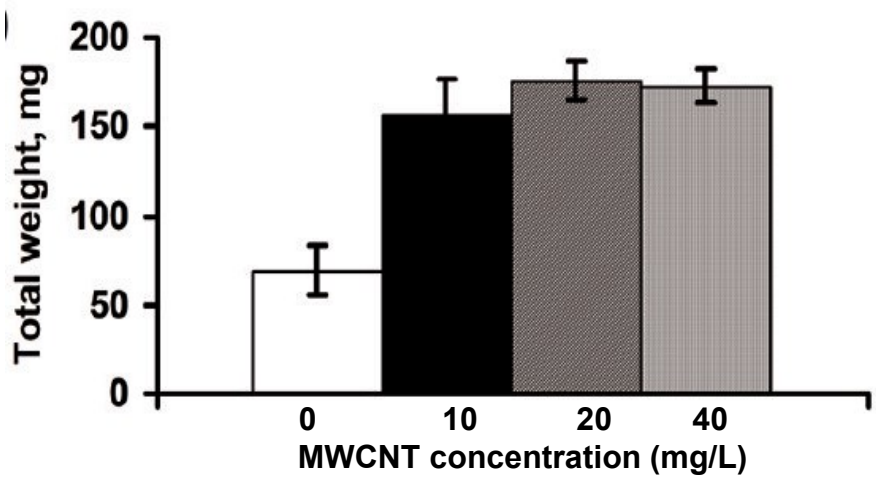
Tomato plantlets after 27 days in hydroponic culture with MWCNT



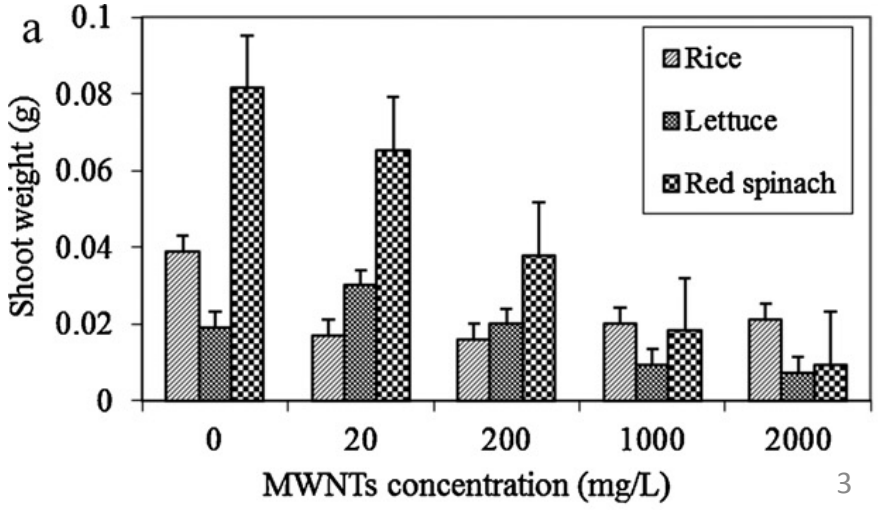
MWCNT concentration (mg/L)

Red spinach plantlets after 14 days in hydroponic culture with MWCNT

MWCNT concentration (mg/L)

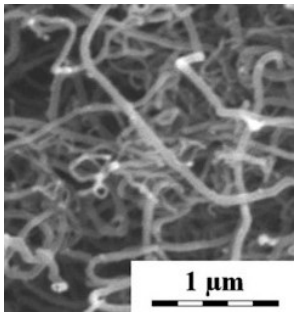


[Khodakovskaya et al., 2009]



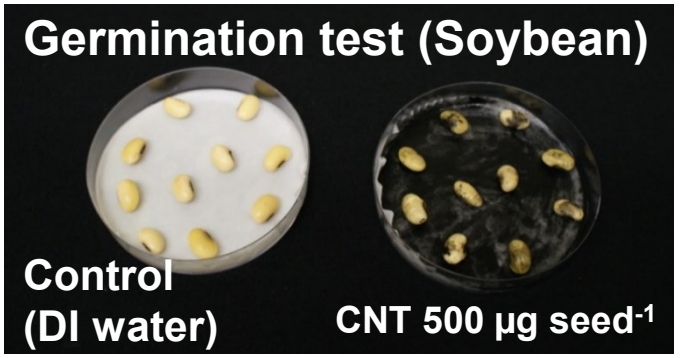
[P. Begum, 2012]

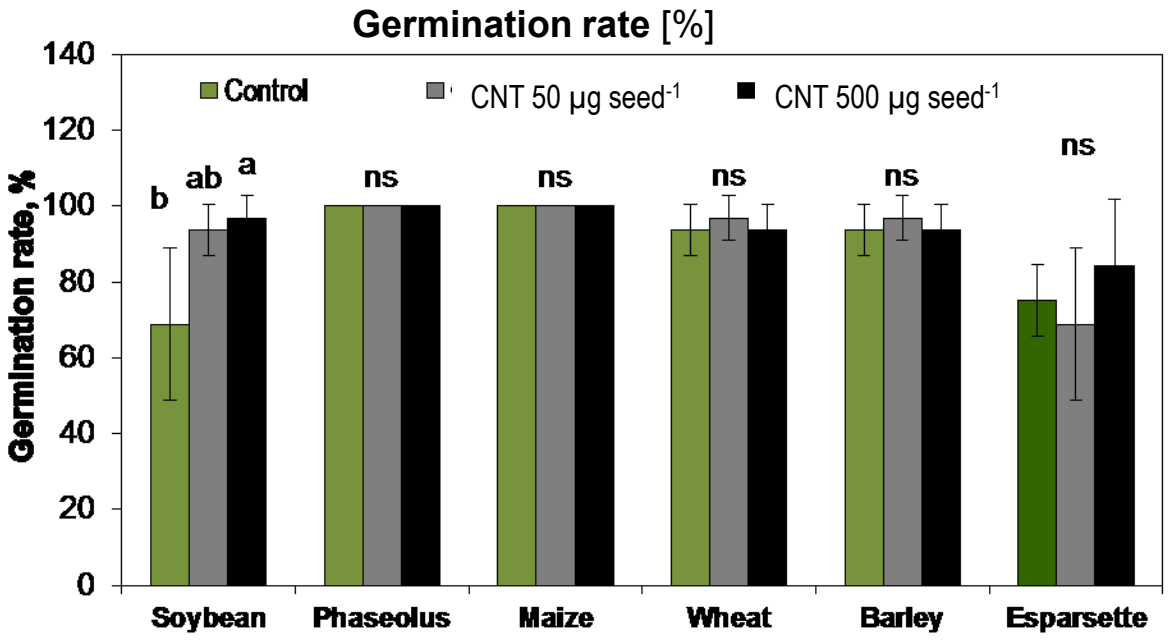
Effect of the MWCNTs on the seed germination

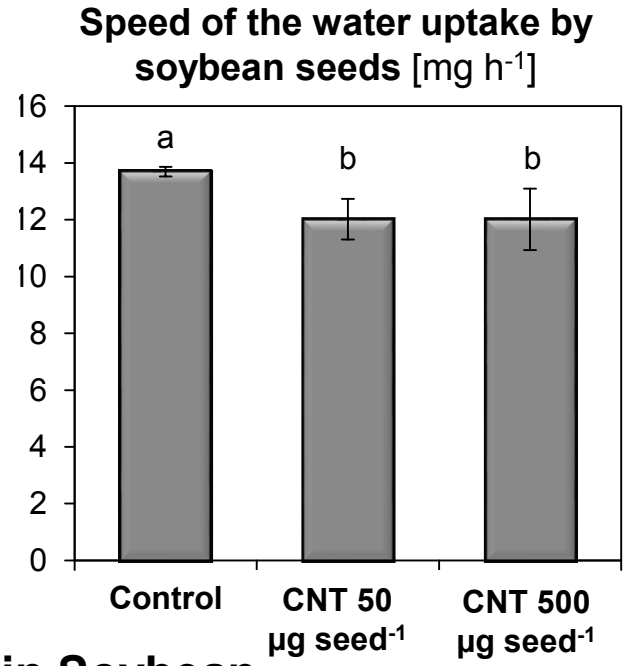


Multi-walled Carbon Nanotubes (CNT)

- external diameter: 20–70 nm
- internal diameter: 5–10 nm
- produced by chemical vapor deposition (CVD)
- purity is above 98%



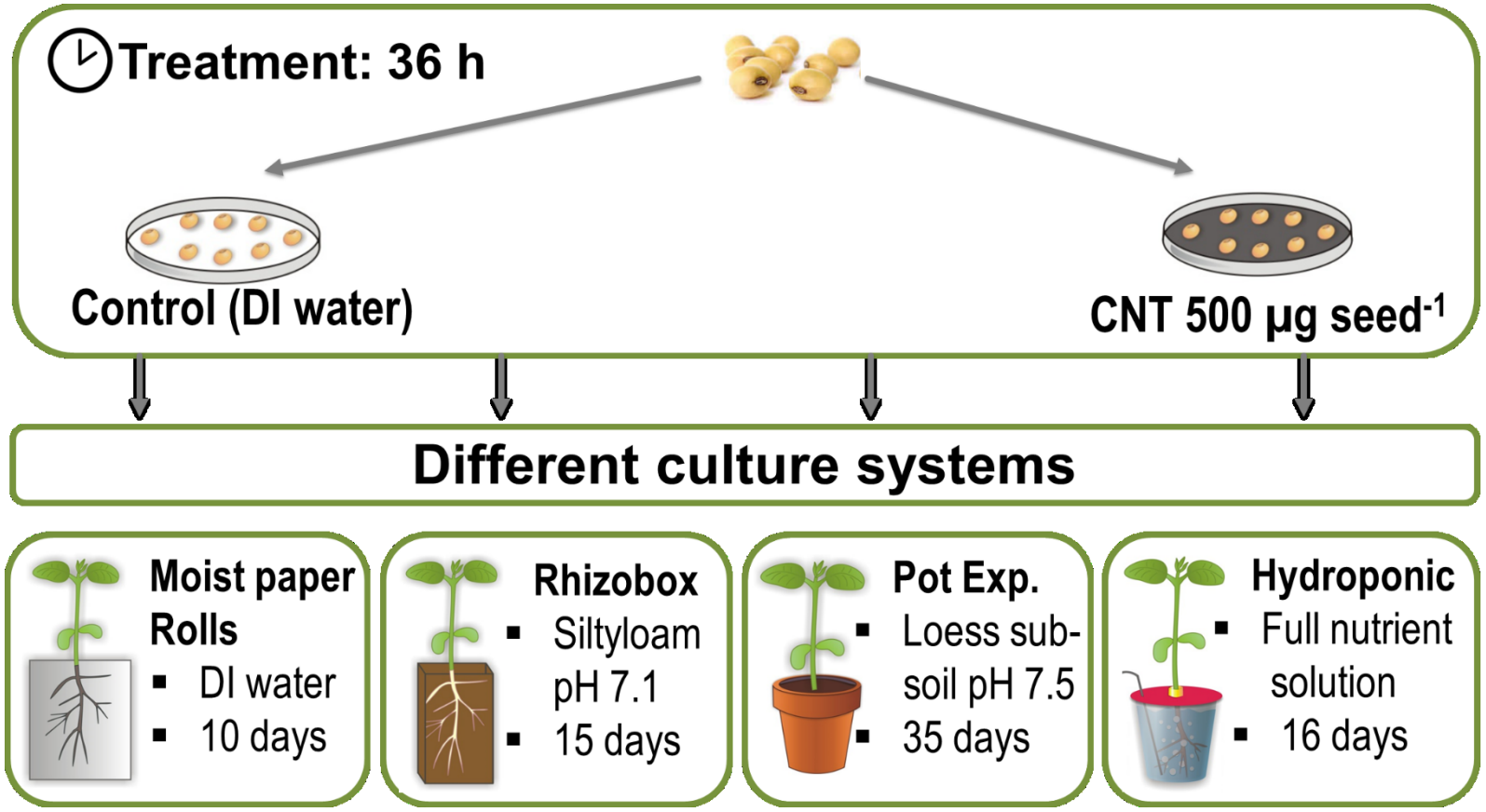




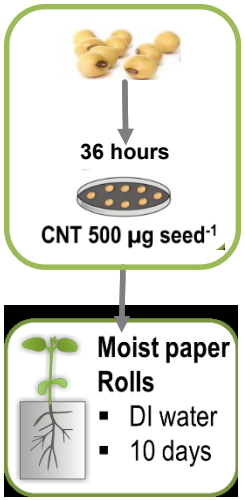
- **CNT-induced stimulation of germination detectable in Soybean**
- **CNT treatments reduced the speed of water uptake in soybean seeds**

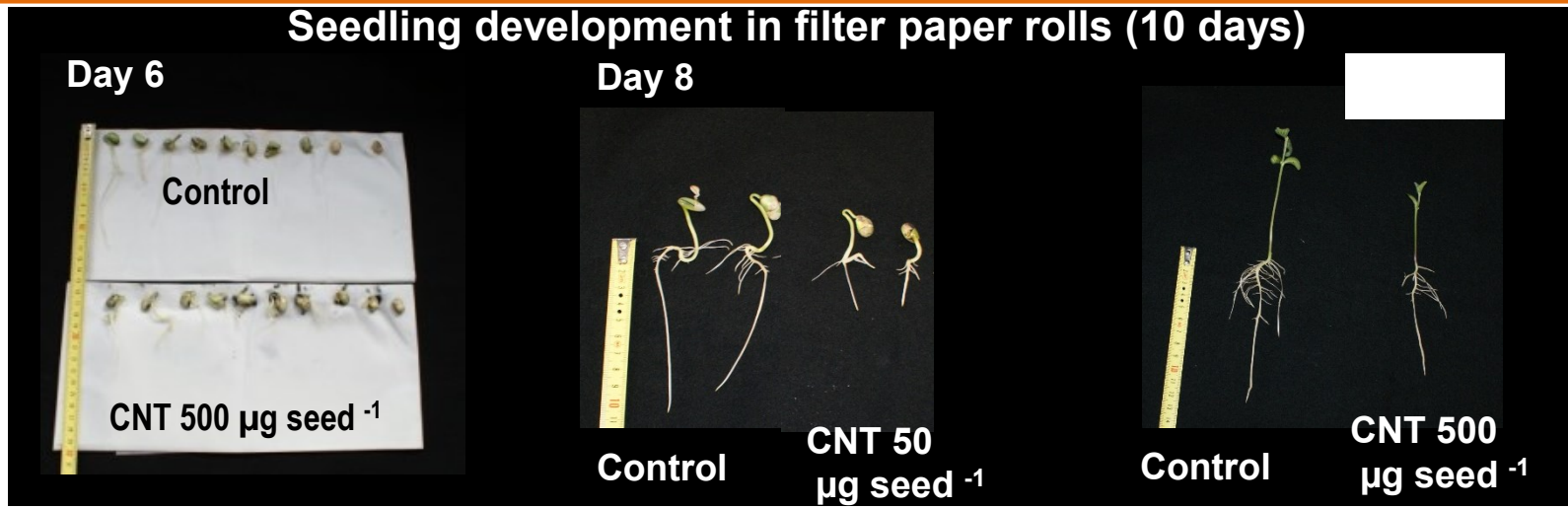
Reduced imbibition damage → Improved germination

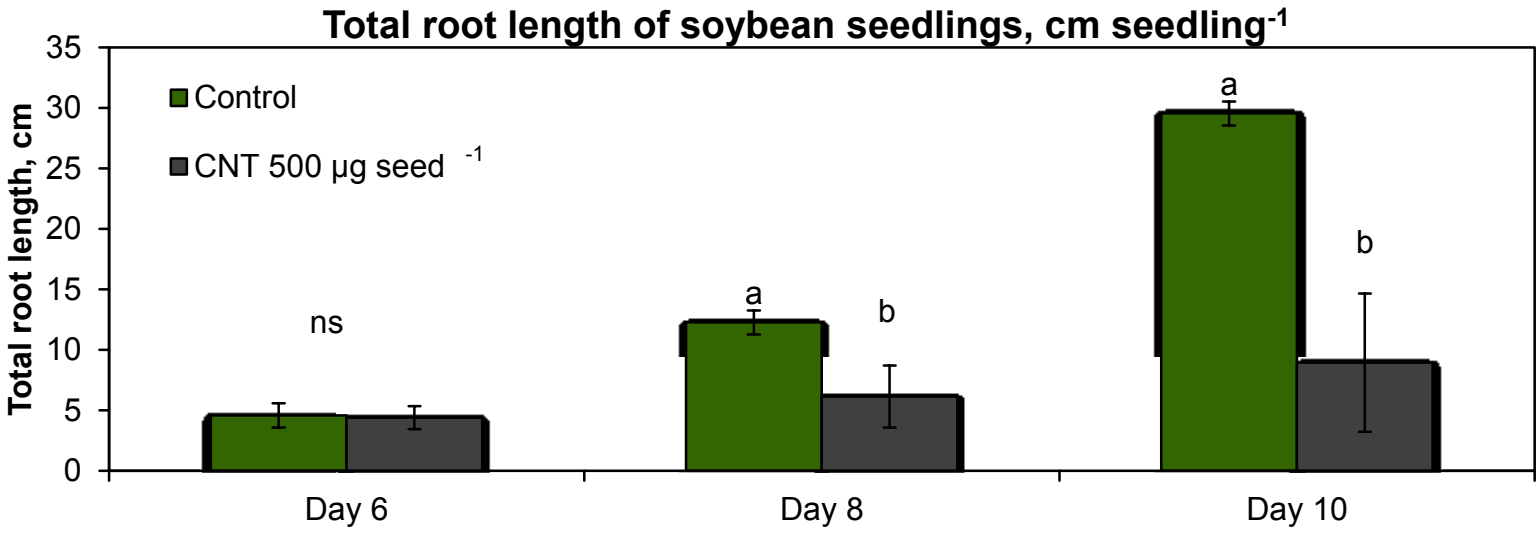
Materials and methods



Effects of CNT on seedling development

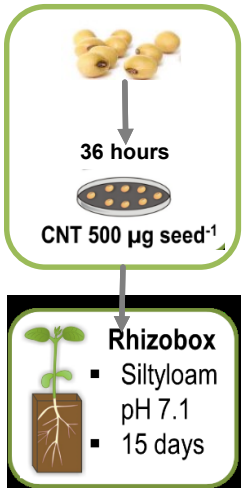






➢ **Despite improved germination, stunted shoot and root growth of CNT-treated soybean seedlings**

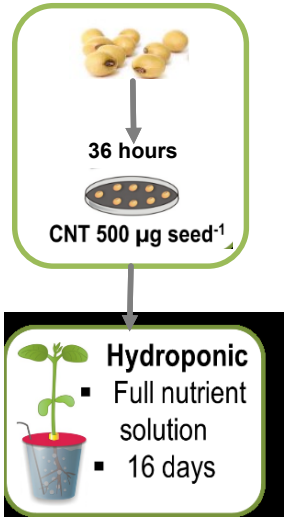
Rhizobox experiment

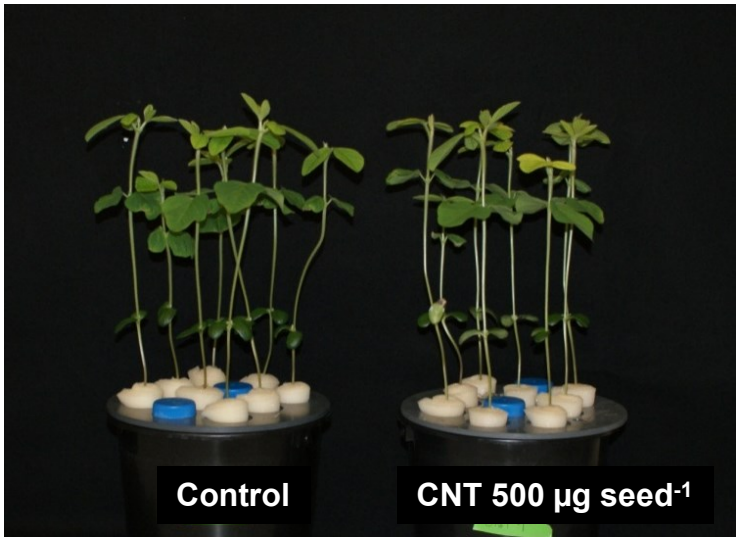


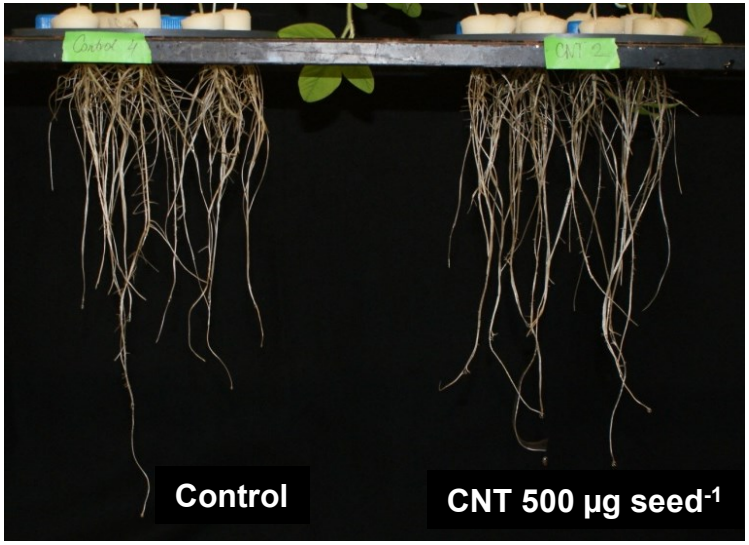
Control	CNT 500 µg seed^{-1}
Root length, cm plant $^{-1}$	
350 cm ^{a}	264 cm ^{b}

➢ **Even short-term CNT treatments** (36 h imbibition) **had long-lasting inhibitory effects on plant growth**

Hydroponic culture





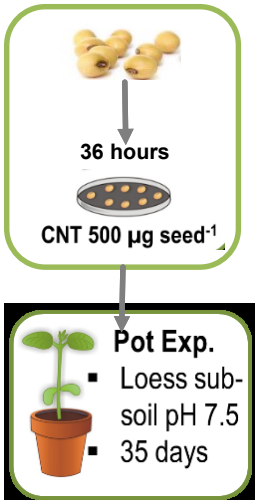


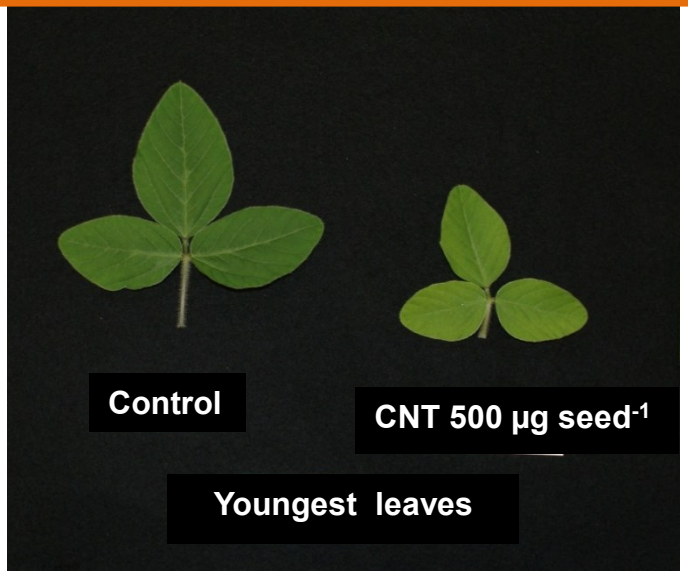
In hydroponic culture, no visual difference between plants treated with CNTs and control plants

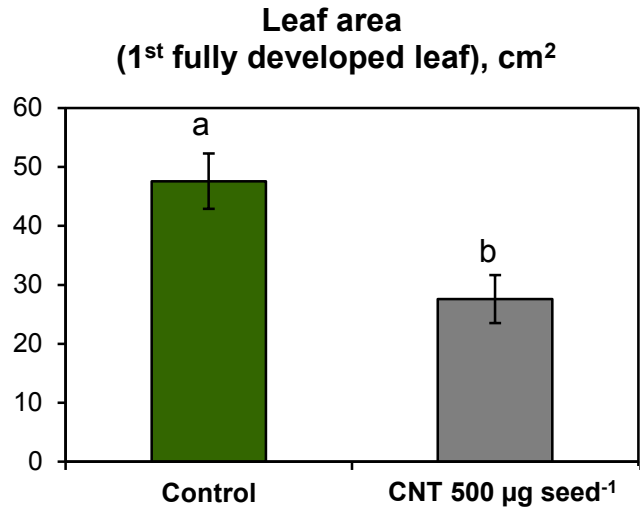
Root growth inhibition is reverted by sufficient supply of easily available nutrients in hydroponics

(Root) growth inhibition during germination or in soil culture may indicate a lack of a specific nutrient not easily available in soil

Pot experiment: Soil culture







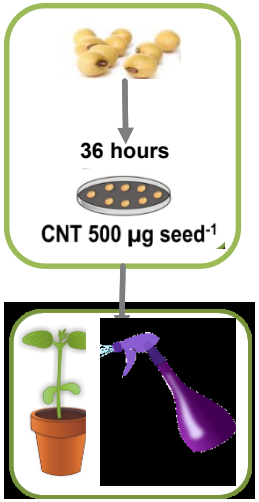
In soybean treated with CNTs:

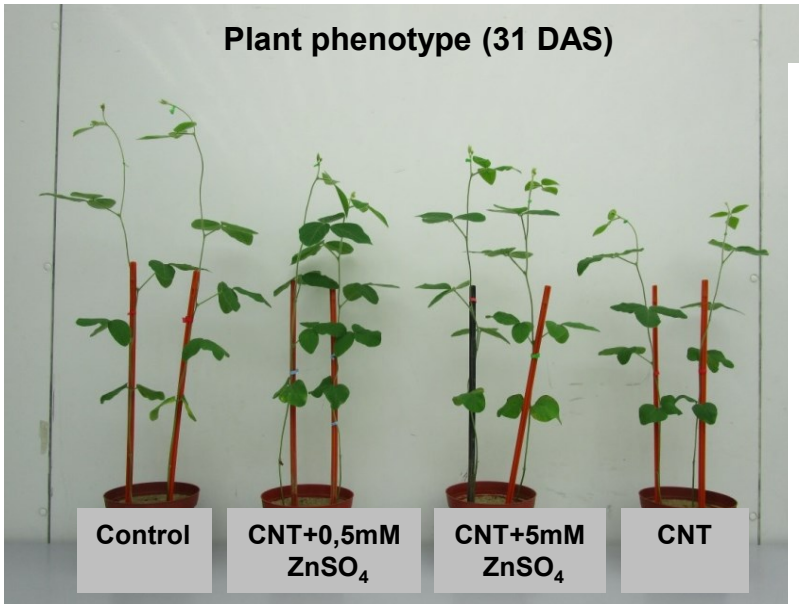
- **Size of the first fully developed leaf significantly reduced**
- **Development of chlorosis**

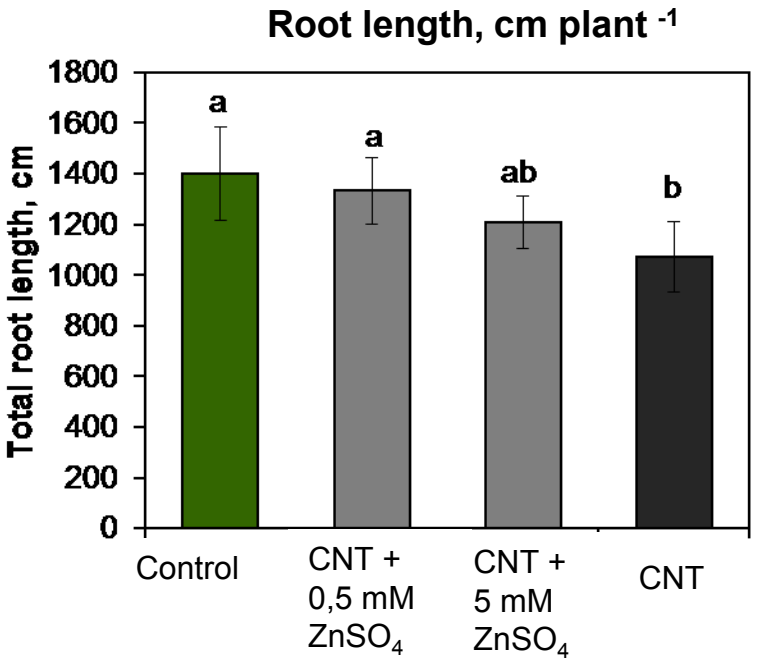
(=„ little leaf syndrome“ characteristic for Zn deficiency)

Zn deficiency confirmed by plant analysis

Foliar Zn application





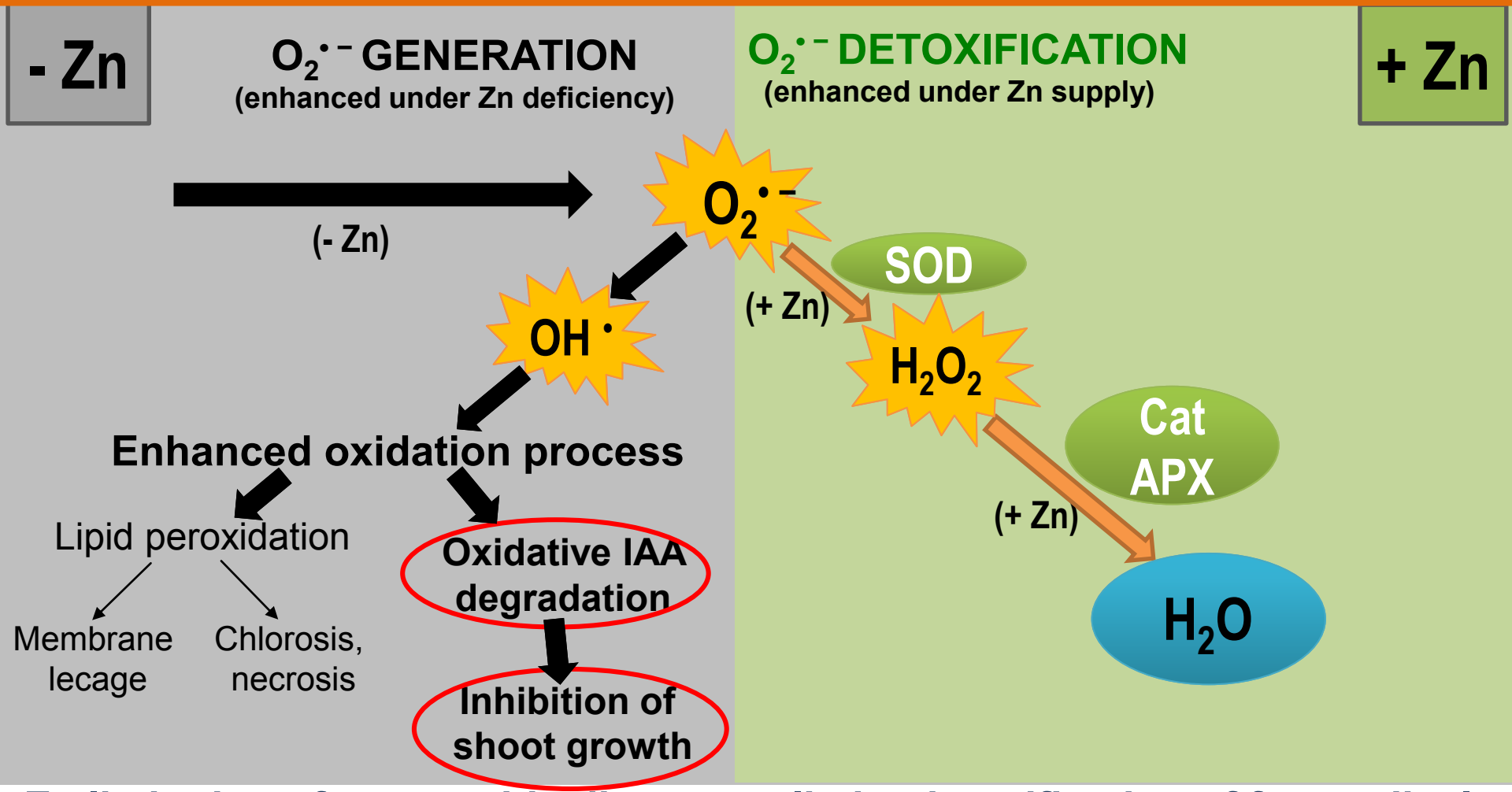


- **Foliar Zn application can mitigate negative effects of CNT on shoot growth and root development of soybean**

- **CNT-treated plants suffer from Zn deficiency!**

Model for Zn deficiency-induced inhibition of shoot and root growth

Modifyed from [Marshner, 2012]



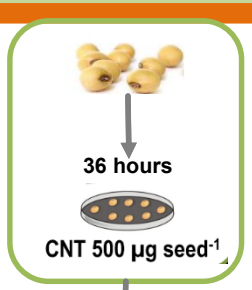
Zn limitation of superoxide dismutase limits detoxification of free radicals

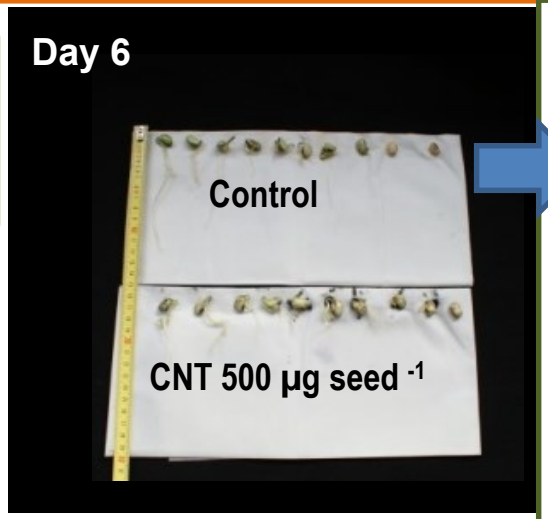
Induction of oxidative degradation of indole-3-acetic acid (IAA)

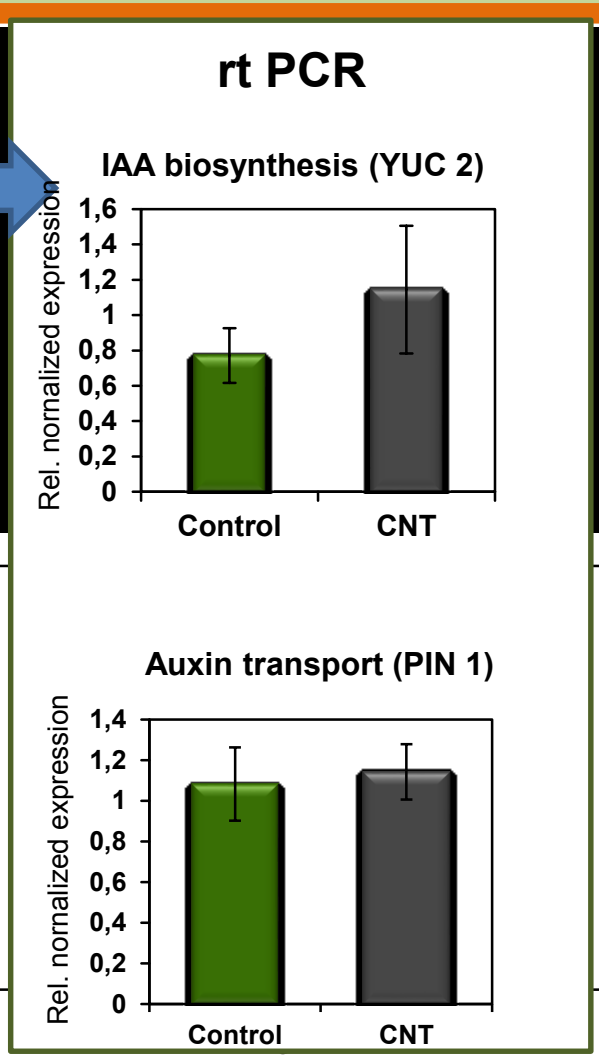
➡ **IAA deficiency**

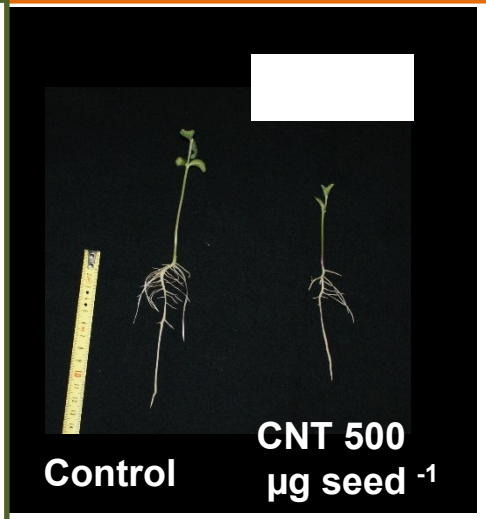
Abbreviations: SOD - Superoxide dismutase; **Cat** – catalase; **APX** - Ascorbate peroxidase; **IAA** - Indole-3-acetic acid

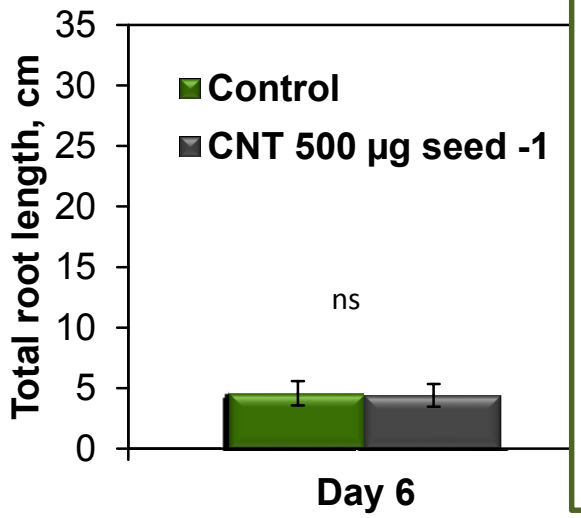
Effects of CNT on seedling development

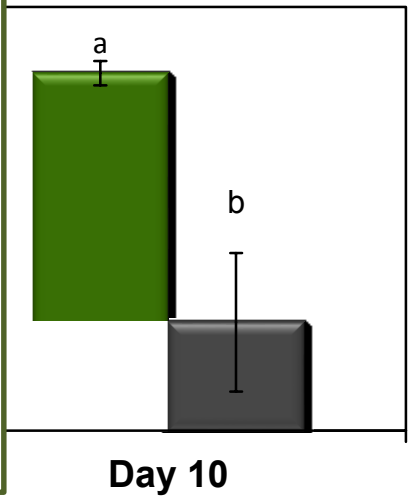












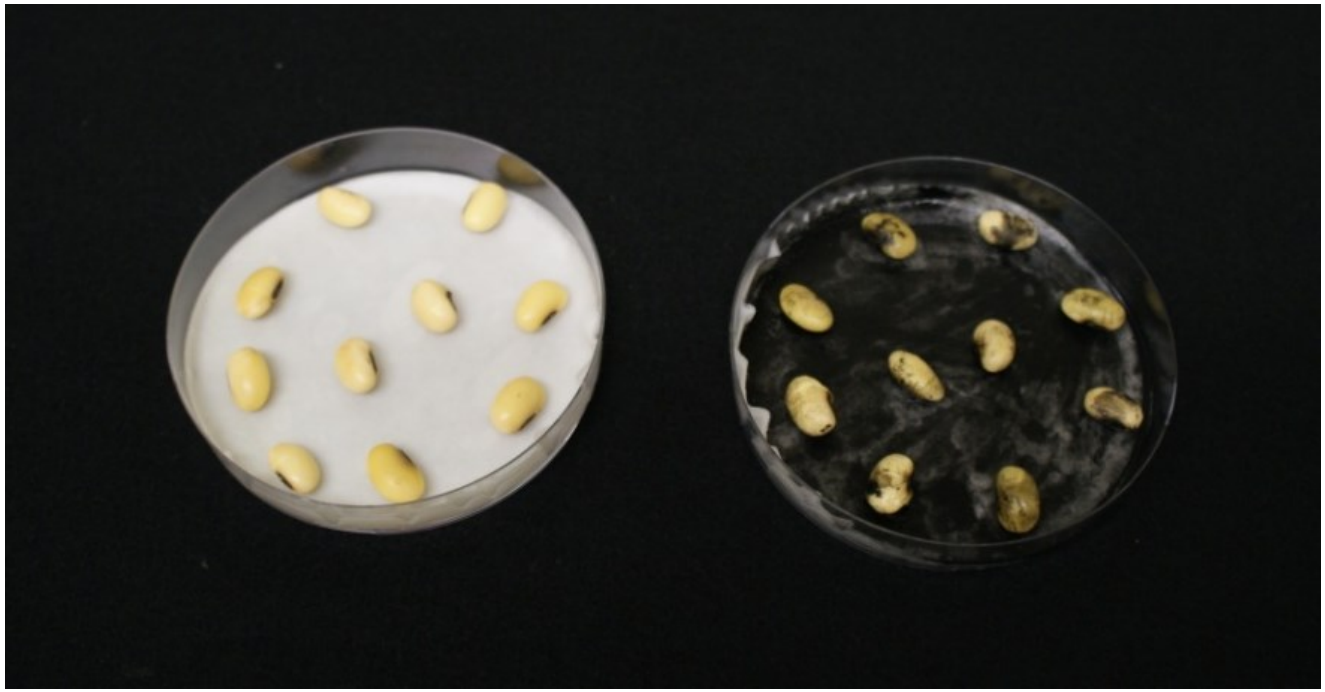
Roots of 6-d old seedlings just prior to appearance of visible damage symptoms were taken for gene expression analysis

No effects on auxin transport

Trend for increased IAA biosynthesis

Compensation for Zn-deficiency induced IAA degradation ?

Phytotoxicity of CNTs

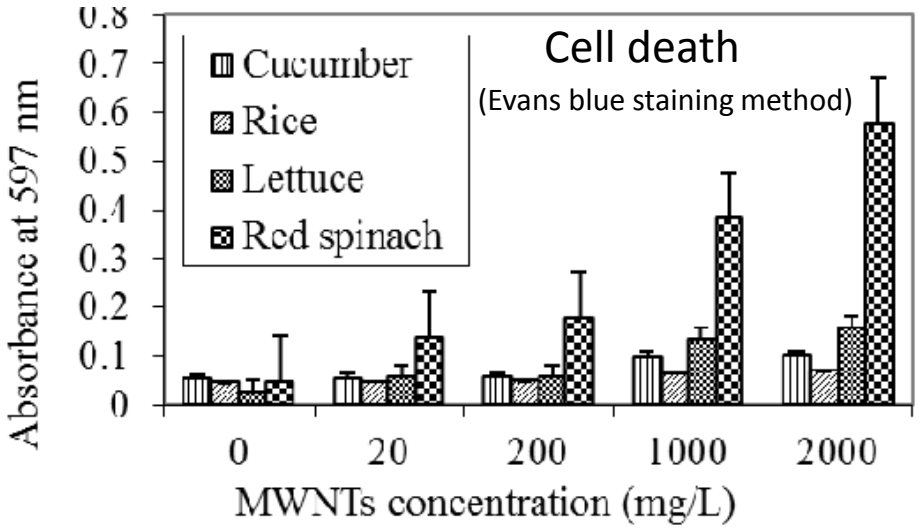


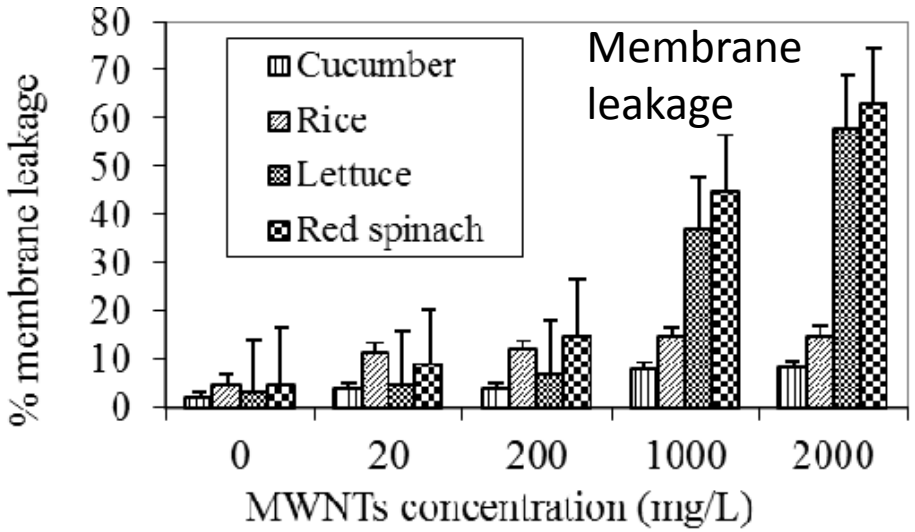
How can a few micrograms of CNTs affect the zinc status and hormonal balances in developing seedlings?

- **Oxidative stress cascade hypothesis**

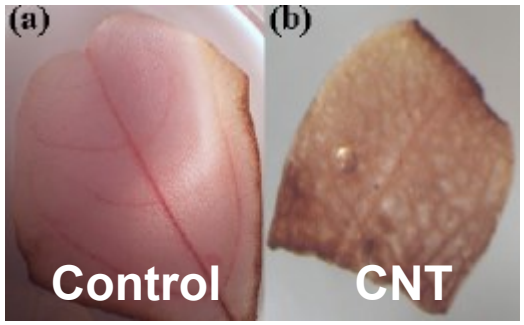
Oxidative stress cascade hypothesis

Seedling roots exposed to CNT treatments in hydroponics (15 d) [Begum et al. 2014]

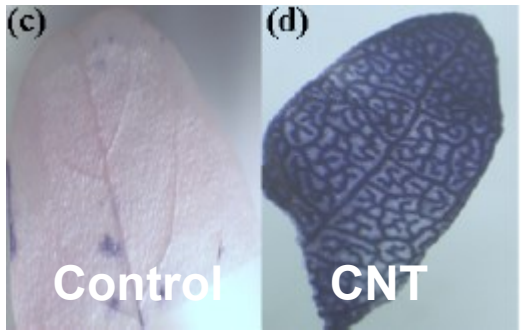




H_2O_2 generation (brown) In leaves



Staining using the 3–3'-diaminobenzidine (DAB)

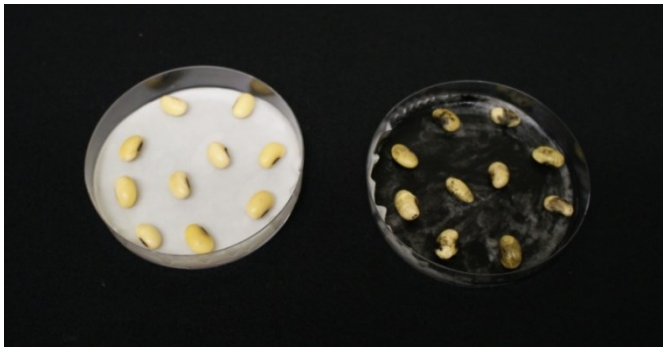


Staining using the NBT

Superoxide Radical formation (blue) in leaves

➢ **Not only Zn deficiency, - also CNT treatments can induce oxidative damage**

Oxidative stress cascade hypothesis



CNT seed treatments induce oxidative damage in germinating seeds

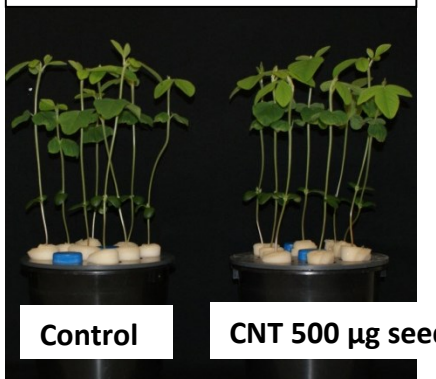
→ Impaired mobilisation/translocation of Zn seed reserves → Zn deficient embryos

→ Zn/auxin-deficient seedlings
→ Stunted root and shoot growth

High Zn availability: nutrient solution or foliar Zn appl.

Recovered plant growth

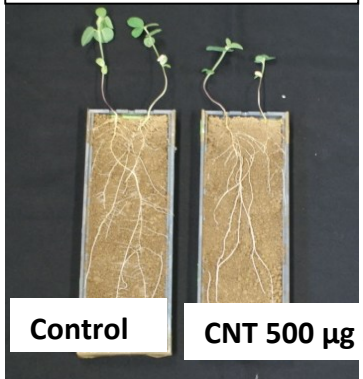
Hydroponic exp. (14 d)



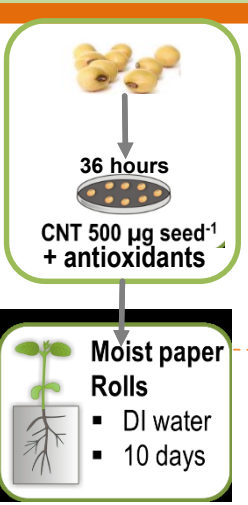
Low Zn availability: soil culture

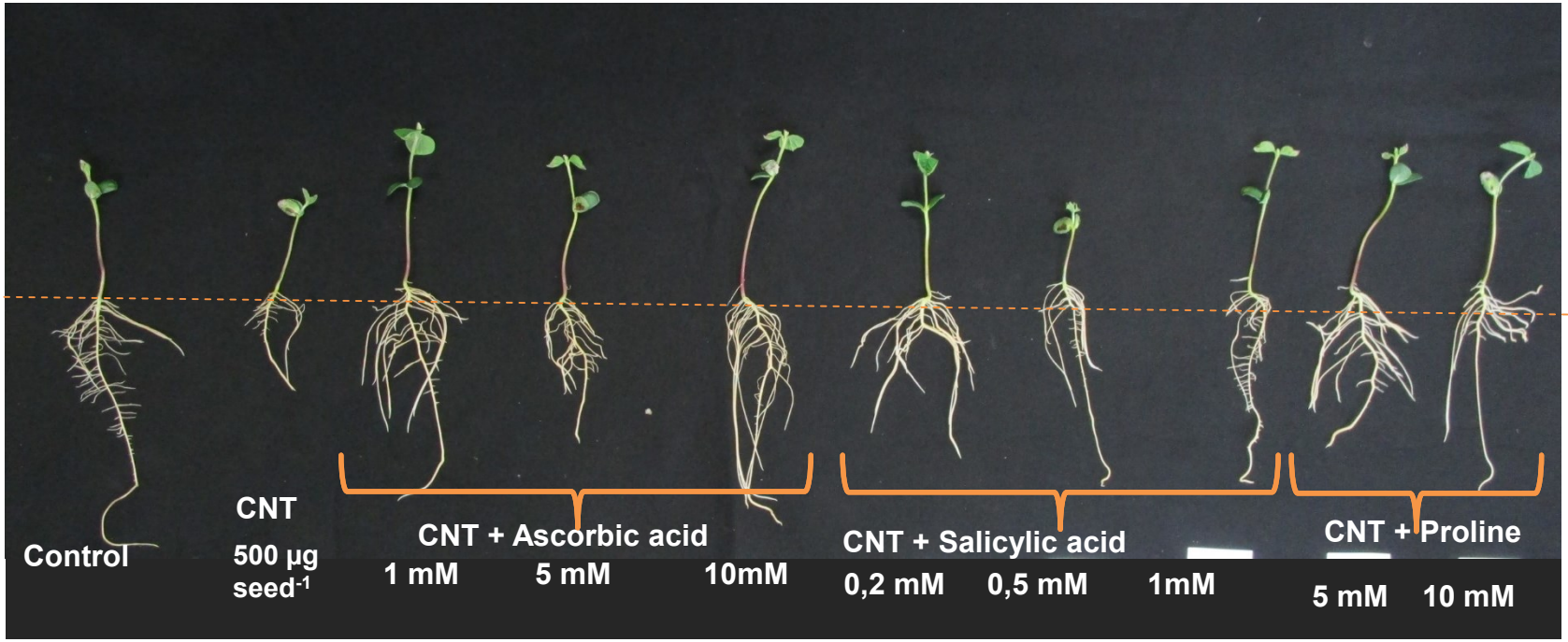
Stunted plant growth

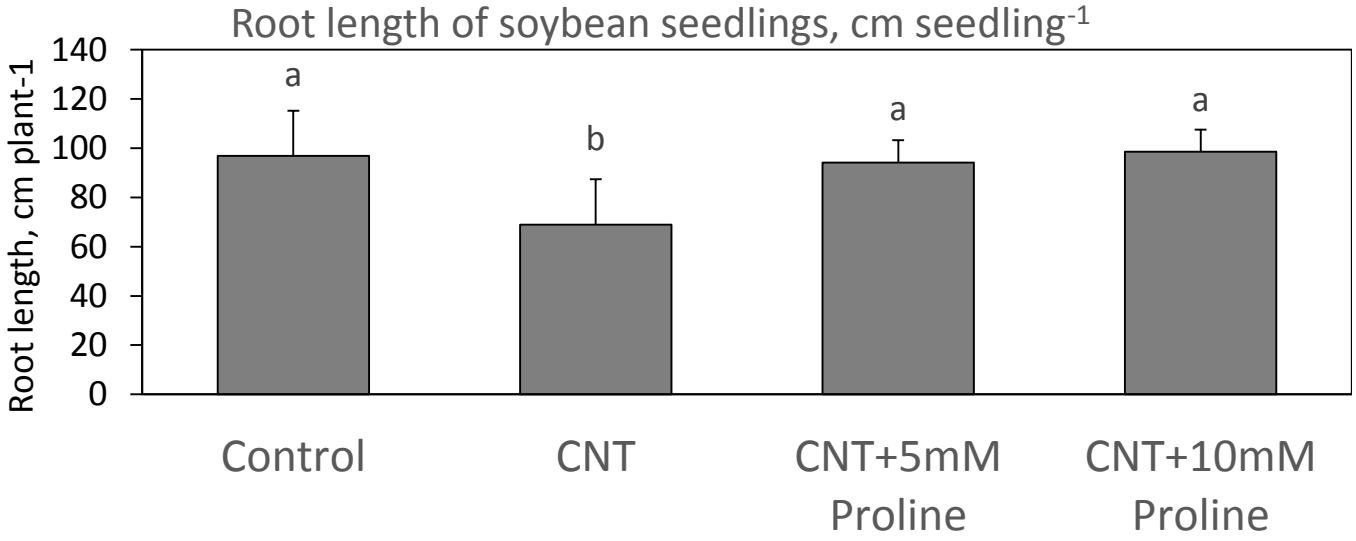
Rhizobox exp. (16 d)



Antioxidants application







Summary

- After short-term seed CNT treatment further seedling development in nutrient deficient substrats is negatevly affected

- Stunted growth is coupled with Zn deficiency and upregulation of auxin biosynthesis while auxin transport is not affected

- Plants can recover if the nutrients are easily available (nutrient solution, foliar Zn application)

- CNT treatments can induce oxidative damage in the seeds presumably trigger cascade of oxidative degradation of indole-3-acetic acid which filally results in stunted growth (confirmed by antioxidant application)

References

Zheng, Y et al. (2014) Distribution Analysis of Nanoparticle Size by Small Angle X-ray Scattering. In International Journal of Theoretical and Applied Nanotechnology. 1 (1), pp. 124-133. DOI: 10.11159/ijtan.2012.019

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Khodakovskaya, Mariya; Dervishi, Enkeleda; Mahmood, Meena; Xu, Yang; Li, Zhongrui; Watanabe, Fumiya; Biris, Alexandru S. (2009): Carbon Nanotubes Are Able To Penetrate Plant Seed Coat and Dramatically Affect Seed Germination and Plant Growth. In ACS Nano 3 (10) pp 3221–3227.

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Thank you for attention!