

# Effects of seed priming and foliar application of Se nanoparticles in the germination, seedling growth, and reproductive stage of tomato and maize

Ezequiel García-Locascio<sup>1</sup>, Edgardo I. Valenzuela<sup>1</sup>, and Pabel Cervantes-Avilés<sup>1\*</sup>

<sup>1</sup>Tecnologico de Monterrey. School of Engineering and Sciences. Puebla, México.

**Abstract.** Sustainable approaches, such as nanotechnology-based strategies, are being researched to increase the productivity of crops. This study aimed to evaluate the effects of nanoprimering with selenium nanoparticles (Se NPs) for tomato (*Solanum lycopersicum*) and maize (*Zea mays*). Additionally, the impact of Se NPs delivered through a foliar application during the reproductive stage of both crops was evaluated. The results showed that Se NPs inhibited the germination rate in maize by up to 100 % but increased the germination rate and vigor index in tomato by 50 and 208 %, respectively (at 10 ppm of Se NPs). Chlorophyll and proline contents were significantly affected in both crops (tomato and maize), suggesting that Se NPs activated the stress defense systems. In contrast, the foliar application of Se NPs on tomato in the reproductive stage increased the length and width of the stem by 10.8 and 4.3 %, respectively. The chlorophyll content increased by 30.8 % at 10 ppm of Se NPs. Additionally, the proline content of reproductive maize plants increased by up to 1303.6 %, indicating significant stressful conditions caused by the exogenous delivery of Se NPs. These results suggested that nanoprimering seeds and foliar application of Se NPs positively impacted tomato. Conversely, the germination of maize seeds and the development of reproductive plants were severely inhibited by Se NPs.

## 1 Introduction

Tomato and maize are two of the most important crops worldwide and are essential to satisfy the global food demand. Tomato is the 2<sup>nd</sup> most crucial vegetable worldwide, and maize is the most important cereal crop [1], [2]. In plants, Selenium (Se) is a nonessential but beneficial element and has been used as a growth promoter in different crops [3], [4]. Se can be an essential micronutrient incorporated in metabolic pathways and nature, and it can be found in combination with nonmetals, metals, and the ionic or nanoparticulate form [5], [6], [7].

Recent studies have focused on the effects that nanoparticles (NPs) of different chemical nature have on crops [8]. Two of the most critical delivery systems of metallic and non-metallic NPs include seed priming and foliar applications [9], [10]. Seed priming is the delivery process of NPs into the seed coat, where the NPs can be internalized and impact the germination positively or negatively. In contrast, foliar delivery involves applying the NPs directly into the foliage as foliar spray [11]. Se NPs have been previously used in foliar applications and seed priming experiments in different crops, including tomato. However, most studies have focused on the effects of Se NPs in the alleviation of biotic

---

\* Corresponding author: [pabel.cervantes@tec.mx](mailto:pabel.cervantes@tec.mx)

(phytopathogens) and abiotic (hyperthermia) stress with both standard delivery systems used, seed priming & foliar application [12], [13].

This work aimed to evaluate the effects of Se NPs on the germination of maize and tomato, as well as their early seedling growth. And also, to evaluate the effects on the reproductive stages of maize and tomato as a function of increasing concentrations of Se NPs (1, 10, and 50 ppm) delivered through seed priming and foliar application in the reproductive stage in plants without induced stress conditions.

## **2. Materials and methods**

### **2.1 Germination essays**

A stock solution of elemental Se NPs with a primary particle size of 50 nanometers (nm) was acquired from ID Nano. Se NPs were dispersed by ultrasonication at 185 W for 30 min and sterilized for 60 min by ultraviolet radiation. The experimental concentrations of 1, 10, and 50 parts per million (ppm) were selected due to the positive effects previously reported for tomato, maize, and other crops [5], [12], [13]. Tomato and maize seeds were selected, sterilized, and thoroughly washed with MilliQ water. 20 seeds per treatment and crop (160 seeds in total) were introduced in their corresponding treatment and they were shaken vigorously for 3 h at 250 rpm. MilliQ water was added as a control to seeds in Petri dishes, which were placed in an incubator with a controlled environment (humidity and temperature), and the germination trays had coconut fiber as substrate and were placed in natural conditions (non-controlled moisture and temperature). After 7 days, measurements of the germination parameters were conducted according to the literature [11], [14]. The parameters measured were germination rate, germination potential, mean germination time, germination index, and vigor index.

### **2.2 Seedlings growth**

After 21 days, the length of maize and tomato seedlings germinated from nanoprimed seeds was measured with a scale (0 – 30 cm), and the fresh weight was obtained with an analytical balance (Ohaus Adventurer). Furthermore, the total chlorophyll content was determined spectrophotometrically according to the standard destructive method of Wintermans & De Mots [15]. The proline content was also calculated spectrophotometrically with the standard method of Bates [16].

### **2.3 Reproductive stage experiments and foliar application**

Maize and tomato plants in their reproductive stage were selected for treatment with Se NPs to analyze the effects of Se NPs in reproductive plants. One foliar application of 1, 10, and 50 ppm of Se NPs delivered the treatments. To this purpose, 20 reproductive plants of each crop were selected and treated with 100 mL of the solution with each concentration. The parameters previously described were determined after the first production of tomato fruits and maize cobs. The total length of the stem was recorded with a measuring tape, while the width of the stem and the width of maize cob and tomato fruit were measured with a Vernier caliper. Furthermore, the total chlorophyll and proline contents were assayed according to the standard methods previously described.

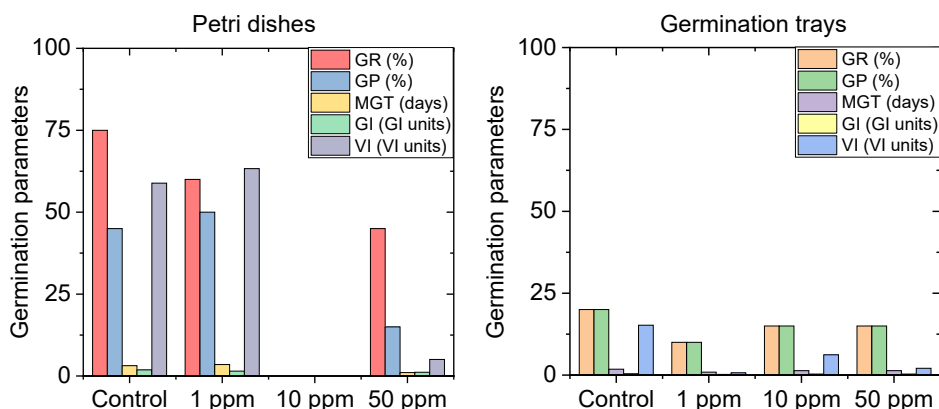
## 2.4 Statistical analyses

The total length and fresh weight of seedlings, total chlorophyll and proline content in seedlings and reproductive plants, the stem length and width in reproductive plants, as well as maize cob and tomato fruit width were statistically analyzed using one-way analysis of variances (ANOVA) with Sigma Plot 11 Software. The data were graphed using Origin Pro 2023b. Multiple comparisons versus the control group were conducted with the Holm-Sidak method; significant differences are based on a probability of  $p < 0.05$ . Each result was obtained with a mean of 4 replicates, the  $\pm$  standard error is represented with vertical lines with caps, and significant differences are represented by “\*.”

## 3 Results and discussion

### 3.1 Germination of maize and tomato seeds primed with Se NPs

The results showed that nanoprimered tomato and maize seeds are either positively or negatively (inhibited) affected by Se NPs. The germination of maize seeds was severely inhibited with all treatments (Fig. 1).

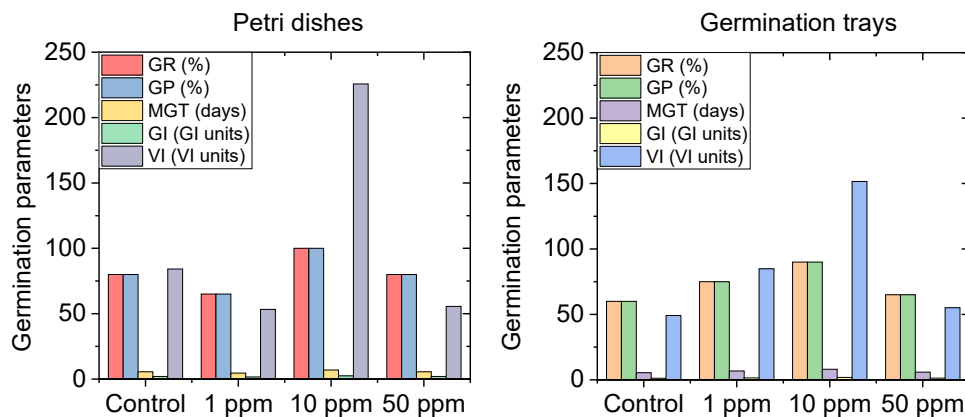


**Fig. 1.** Germination of maize seeds primed with Se NPs. The left graphic represents Petri dishes, and the right represents trays. (GR) Germination rate. (GP) Germination potential. (MGT) Mean germination time. (GI) Germination index. (VI) Vigor index.

All Se NPs concentrations applied reduced the germination rate and germination indexes between 20 – 100 % and 25 – 50 % in Petri dishes and trays, respectively. The germination potential and the mean germination time were also reduced between 66.7 – 100 % with 10 and 50 ppm in Petri dishes, with slight increases of 11.1 % with 1 ppm, whereas in trays, the inhibition ranged from 25 – 50 % with all treatments. Furthermore, the vigor index was reduced between 91.4 and 100 % with 50 and 10 ppm, respectively, in Petri dishes, with a slight increase of 7.52 % for 1 ppm, whereas in trays, the reductions ranged from 59.4 – 95.5 % with all treatments (Fig. 1).

These results exhibit the negative impact of Se NPs in the germination of maize seeds, which is in accordance with studies using NPs of different natures in maize germination and growth [14], [17], [18]. Furthermore, the germination of nanoprimered maize seeds in Petri dishes was less impacted than in trays, showing slight increases in some parameters at 1 ppm of Se NPs. This is expected due to the controlled environment in Petri dishes, where relative humidity and temperature were kept constant at 95 % and 25°C, respectively.

In contrast with the results obtained in nanoprimed maize seeds, the germination of tomato seeds primed with 10 ppm of Se NPs was positive. The results showed that from the parameters measured, the germination rate, germination potential, mean germination time, and germination index increased between 25 and 50 % in Petri dishes and trays, respectively. In comparison, the vigor index increased between 168.1 and 208 % in Petri dishes and trays, respectively, with 1 and 10 ppm of Se NPs (Fig. 2).



**Fig. 2.** Germination of tomato seeds primed with Se NPs. The left graphic represents Petri dishes, and the right represents trays. (GR) Germination rate. (GP) Germination potential. (MGT) Mean germination time. (GI) Germination index. (VI) Vigor index.

The results obtained with nanoprimed tomato seeds are in line with the positive impact of Se NPs in tomato previously reported [12], [13], [19], where the applications have focused on Se NPs' effects in alleviating biotic (phytopathogens) and abiotic (hyperthermia) stress. Furthermore, the controlled environment explains the increased germination rate of Petri dishes as compared to trays, where the temperature (around 19.9°C) and humidity were significantly lower [20].

The differences in the germination of maize and tomato seeds primed with Se NPs might rely on the different vasculature and photosynthetic pathways of each plant species [21]. While tomato is a dicotyledonous plant with a C3 photosynthetic metabolic pathway, maize is a monocotyledonous plant with a C4 photosynthetic metabolic pathway [22]. Such differences have been previously identified to shape the effects of NPs, as observed with CeO<sub>2</sub> NPs in tomato and maize, where the translocation was more efficient in the dicotyledonous species (tomato) [23].

The mechanisms of the improvement or inhibition caused by Se NPs and other types of NPs are still widely unknown; however, other studies have proposed that germination might be affected due to the NPs creating nanopores during their internalization into the seed [24]. Once internalized, the NPs can impact positively or negatively the metabolic pathways of the seedlings and the biogenesis of the organelles within the cells, ultimately affecting the growth of the seedlings [25]. To better understand the effects of Se NPs, the growth of germinated tomato and maize seedlings was evaluated.

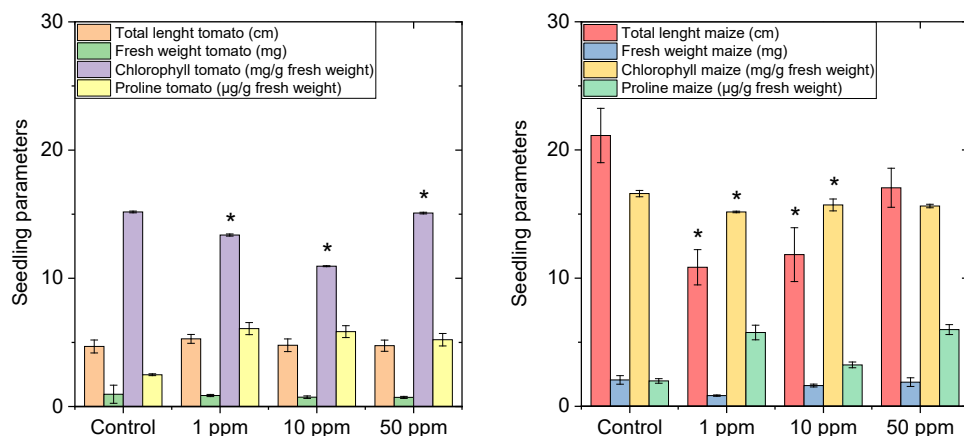
### 3.2 Growth of maize and tomato seedlings

The growth of tomato seedlings germinated from Se NPs nanoprimed tomato seeds was slightly improved in all treatments. The total length increased between 1.3 and 12.6 %. In contrast, all treatments reduced the fresh weight between 10.3 and 25.6 %. Meanwhile, in maize growth, inhibition was severe, reducing the total length of the seedlings between 19.3

and 48.6 % and the fresh weight between 8.4 and 59.4 %, with 1 ppm causing the highest inhibition.

The total chlorophyll content was significantly reduced in tomato, ranging from 0.6 – 27.9 %. Additionally, the total chlorophyll content was slightly reduced in maize, where the decrease fluctuated between 5.3 – 8.6 %. Furthermore, the proline content was significantly increased in tomato and maize seedlings. The increases in tomato ranged between 110.2 and 145.1 %, while in maize, they varied from 63.2 to 202.3 %.

These results show that nanoprimering tomato and maize seeds with Se NPs can negatively impact the growth of the seedlings, notwithstanding the increased germination and total length of the tomato. Moreover, the negative results obtained in the germination of maize, such as inhibition, continued throughout the seedling growth but were less severe. Still, the proline content increased in both crops, indicating induced-stress conditions (Fig. 2 & Fig. 3).



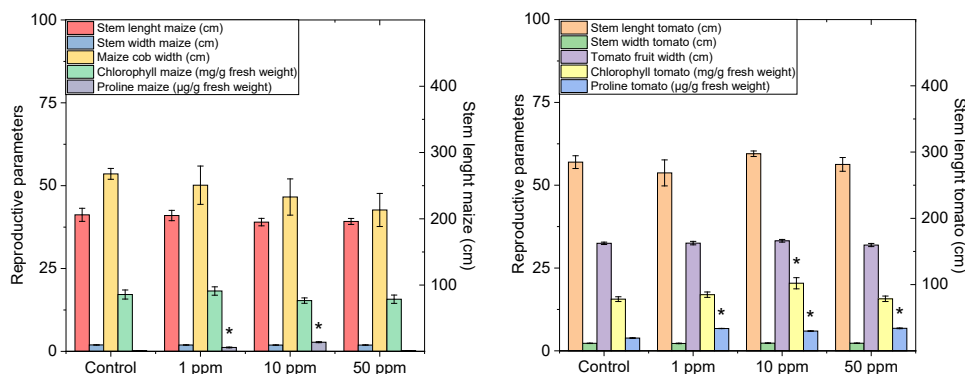
**Fig. 3.** Seedling growth parameters of maize and tomato primed with Se NPs. The left graphic represents maize, and the right represents tomato. Total length (cm). Fresh weight (mg). Total chlorophyll content (mg/g fresh weight). Proline content (mg/kg fresh weight). Multiple comparisons versus the control group were conducted with the Holm-Sidak method; significant differences are based on a probability of  $p < 0.05$ . Each result was obtained with a mean of 4 replicates, the  $\pm$  standard error is represented with vertical lines with caps, and significant differences are represented by “\*.”

Monitoring the proline content is important due to proline being associated with plants exposed to stressful conditions [26], [27]. Proline is a vital osmolyte involved in the antioxidative defense system, and the overproduction of proline maintains the osmotic balance by preventing electrolyte leakage [28]. Also, chlorophyll is an essential photosynthetic pigment synthesized from the same pathway as proline, which is glutamate [28], [29]. The mechanisms in which Se NPs affect the total chlorophyll and proline contents are still unknown; however, we hypothesize that upon internalization by the seeds, the Se NPs might affect the metabolic pathway in which the cells biosynthesize chlorophyll and proline. Furthermore, the significant increases in proline might explain the enhancement in the total length of tomato seedlings [26], [30].

### 3.3 Effects of Se NPs in the reproductive stage of maize and tomato plants

The growth of maize and tomato plants in the reproductive stage mainly remained unaffected. However, slight increases in the stem length and width (10.8 and 4.3 %, respectively) were recorded at 10 ppm of Se NPs. Furthermore, the width of tomato fruits increased by up to 2.2 % with the same treatment. Meanwhile, in maize plants, the length and width of the stem

were reduced by as much as 4.9 and 1.7 %, respectively, at 10 ppm concentration. Moreover, the width of the maize cob was decreased by 8.4 % with 50 ppm of Se NPs (Fig. 4).



**Fig. 4.** Reproductive stage parameters of maize and tomato treated with a foliar application of Se NPs. The left graphic represents maize, and the right represents tomato. Stem length (cm). Stem width (cm). Fruit and cob width (cm). Total chlorophyll content (mg/g fresh weight). Proline content (mg/kg fresh weight). Multiple comparisons versus the control group were conducted with the Holm-Sidak method; significant differences are based on a probability of  $p < 0.05$ . Each result was obtained with a mean of 4 replicates, the  $\pm$  standard error is represented with vertical lines with caps, and significant differences are represented by “\*.”

The chlorophyll content of maize plants increased by 6.2 % with 1 ppm. However, it decreased between 8.3 – 10.7 % with 50 and 10 ppm, respectively. Moreover, the proline content increased by 498.8 and 1303.6 % with 1 and 10 ppm, while 50 ppm only increased it by 7.2 %. In contrast, the total chlorophyll content of tomato plants increased between 0.6 – 30.8%, with 10 ppm achieving the best results. Whereas the proline content of reproductive tomato plants also increased in a range of 54.6 – 75.9 %, the lowest increase recorded at 10 ppm (Fig. 4). In a previous study, foliar-delivered Se NPs in the reproductive stage of wheat (*Triticum aestivum*) at a concentration of 5 g Se NPs/Ha had positive results on the productivity [5]. Furthermore, 60 g Se NPs/Ha had positive results in the productivity of maize in field conditions [6]. However, the concentrations were significantly lower than those in the present study. The negative impact of foliar-delivered Se NPs was not as significant in reproductive maize plants as the seed priming. However, the proline content indicated that plants had abiotic stress conditions caused by the Se NPs; the internalization of Se NPs could explain this by damaging the tissues, which could cause membrane lysis and cell death [24]. In tomato, the results were positive in both the germination and the growth of reproductive plants with 10 ppm, which could be potentially due to the beneficial effects of Se in the antioxidative system of plants [31], [32], [33].

## 4 Conclusions

In this study, nanoprimering tomato and maize seeds with Se NPs resulted in different outcomes. Se NPs had a positive impact on the germination and growth in the early vegetative and reproductive stages of tomato, with 10 ppm achieving the best results. However, the results in maize showed that Se NPs severely inhibited all parameters with all treatments. We recommend using different concentrations of Se NPs and including more physiological parameters to bridge the knowledge gap between the effects of Se NPs and various crops. Furthermore, the development stage of the crops should also be considered, it appears that Se NPs have a more substantial impact on early seedling growth than in reproductive plants, but more research is essential to discern such differences. Moreover, the photosynthetic

metabolism of crops and their vasculature system are believed to substantially affect the impact of Se NPs, as shown in this study. Therefore, more studies are urgently needed to elucidate the mechanisms of internalization of Se NPs and their effects on plant tissues on deeper levels. Currently, nanotechnology in agriculture remains a promising tool to make agriculture more sustainable, but more research is essential before implementing NPs in commercial agriculture.

EGL would like to thank Tecnológico de Monterrey and CONAHCYT for the scholarship granted (1016970). EGL acknowledges Tecnológico de Monterrey and CAETEC for the facilities to carry out the experiments. PCA thanks to CONAHCYT (330129). This work was funded by Tecnológico de Monterrey, Challenge-Based Research Funding Program, E051-EIC-GI10-A-T8-E.

## References

1. B. K. Singh, P. Trivedi, E. Egidi, C. A. Macdonald, M. Delgado-Baquerizo, Crop microbiome and sustainable agriculture, *Nature Reviews Microbiology*, **18**, 11 (2020). <https://doi.org/10.1038/s41579-020-00446-y>
2. M. A. Altaf *et al.*, Melatonin alleviates salt damage in tomato seedling: A root architecture system, photosynthetic capacity, ion homeostasis, and antioxidant enzymes analysis, *Sci Hort*, **285** (2021). <https://doi.org/10.1016/j.scienta.2021.110145>
3. T. Zhan *et al.*, Chitin combined with selenium reduced nitrogen loss in soil and improved nitrogen uptake efficiency in Guanxi pomelo orchard, *Science of the Total Environment*, **799** (2021). <https://doi.org/10.1016/j.scitotenv.2021.149414>
4. G. Wang, H. Zhang, F. Lai, H. Wu, Germinating Peanut (*Arachis hypogaea* L.) Seedlings Attenuated Selenite-Induced Toxicity by Activating the Antioxidant Enzymes and Mediating the Ascorbate-Glutathione Cycle, *J Agric Food Chem.* **64**, 6 (2016). <https://doi.org/10.1021/acs.jafc.5b05945>
5. D. Delaqua, R. Carnier, R. S. Berton, F. C. A. Corbi, A. R. Coscione, Increase of selenium concentration in wheat grains through foliar application of sodium selenate, *Journal of Food Composition and Analysis*, **99** (2021). <https://doi.org/10.1016/j.jfca.2021.103886>
6. Q. Wang *et al.*, Effects of Different Forms of Selenium Fertilizers on Se Accumulation, Distribution, and Residual Effect in Winter Wheat-Summer Maize Rotation System, *J Agric. Food. Chem.* **65**, 6 (2017). <https://doi.org/10.1021/acs.jafc.6b05149>
7. Z. Pu *et al.*, Selenium and anthocyanins share the same transcription factors R2R3MYB and bHLH in wheat, *Food Chem.* **356** (2021), <https://doi.org/10.1016/j.foodchem.2021.129699>
8. S. M. Zahedi, F. Moharrami, S. Sarikhani, M. Padervand, Selenium and silica nanostructure-based recovery of strawberry plants subjected to drought stress, *Sci. Rep.* **10**, 1 (2020). <https://doi.org/10.1038/s41598-020-74273-9>
9. P. Cervantes-Avilés, X. Huang, A. A. Keller, Dissolution and Aggregation of Metal Oxide Nanoparticles in Root Exudates and Soil Leachate: Implications for Nanoagrochemical Application, *Environ. Sci. Technol.* **55**, 20 (2021). <https://doi.org/10.1021/acs.est.1c00767>
10. S. Talebian, T. Rodrigues, J. Das Neves, B. Sarmiento, R. Langer, J. Conde, Facts and Figures on Materials Science and Nanotechnology Progress and Investment, *ACS Nano*, **15**, 10 (2021). <https://doi.org/10.1021/acsnano.1c03992>

11. M. W. Mazhar, M. Ishtiaq, M. Maqbool, R. Akram, Seed priming with Calcium oxide nanoparticles improves germination, biomass, antioxidant defence and yield traits of canola plants under drought stress, *South African Journal of Botany*, **151** (2022). <https://doi.org/10.1016/j.sajb.2022.11.017>
12. S. V. Gudkov et al., Production and Use of Selenium Nanoparticles as Fertilizers, *ACS Omega*, **5**, 28 (2020). <https://doi.org/10.1021/acsomega.0c02448>
13. S. M. Joshi, S. De Britto, S. Jogaiah, Myco-engineered selenium nanoparticles elicit resistance against tomato late blight disease by regulating differential expression of cellular, biochemical and defense responsive genes, *J. Biotechnol.*, **325** (2021). <https://doi.org/10.1016/j.jbiotec.2020.10.023>
14. C. Gong, L. Wang, X. Li, H. Wang, Y. Jiang, W. Wang, Responses of seed germination and shoot metabolic profiles of maize (*Zea mays* L.) to Y2O3 nanoparticle stress, *RSC Adv.* **9**, 47 (2019). <https://doi.org/10.1039/c9ra04672k>
15. J. F. G. M. Wintermans A. De Mots, 44 S *Biochimica et Biophysica Acta -BBA* 45 224 Spectrophotometric Characteristics Of Chlorophylls a And b And Their Pheophytins In Ethanol (1965). [https://doi.org/10.1016/0926-6585\(65\)90170-6](https://doi.org/10.1016/0926-6585(65)90170-6)
16. R. P. W. & I. D. T. L. S. Bates, Rapid determination of free proline for water-stress studies (1973). <https://doi.org/10.1007/BF00018060>
17. J. Lian et al., Foliar spray of TiO2 nanoparticles prevails over root application in reducing Cd accumulation and mitigating Cd-induced phytotoxicity in maize (*Zea mays* L.), *Chemosphere*, **239** (2020). <https://doi.org/10.1016/j.chemosphere.2019.124794>
18. J. Cao, Y. Feng, S. He, X. Lin, Silver nanoparticles deteriorate the mutual interaction between maize (*Zea mays* L.) and arbuscular mycorrhizal fungi: a soil microcosm study, *Applied Soil Ecology*, **119** (2017). <https://doi.org/10.1016/j.apsoil.2017.04.011>
19. A. Shiriaev et al., Selenium Biofortification Impacts the Tomato Fruit Metabolome and Transcriptional Profile at Ripening, *J. Agric. Food. Chem.* **71**, 36 (2023). <https://doi.org/10.1021/acs.jafc.3c02031>
20. C. B. Rohal, C. R. Adams, C. W. Martin, S. Tevlin, L. K. Reynolds, Seed bank and germination ecology of sub-tropical *Vallisneria americana*, *Aquat. Bot.* **190** (2024), <https://doi.org/10.1016/j.aquabot.2023.103721>
21. Z. Wang et al., Xylem- and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.), *Environ. Sci. Technol.* **46**, 8 (2012). <https://doi.org/10.1021/es204212z>
22. J. Hong, C. Wang, D. C. Wagner, J. L. Gardea-Torresdey, F. He, C. M. Rico, Foliar application of nanoparticles: Mechanisms of absorption, transfer, and multiple impacts, *Environmental Science: Nano*, **8**, 5 (2021). <https://doi.org/10.1039/d0en01129k>
23. E. Spielman-Sun et al., Nanoparticle surface charge influences translocation and leaf distribution in vascular plants with contrasting anatomy, *Environ. Sci. Nano.* **6**, 8 (2019). <https://doi.org/10.1039/c9en00626e>
24. J. Lv, P. Christie, S. Zhang, Uptake, translocation, and transformation of metal-based nanoparticles in plants: recent advances and methodological challenges, *Environmental Science: Nano*, **6**, 1 (2019). <https://doi.org/10.1039/C8EN00645H>
25. A. do E. S. Pereira, H. C. Oliveira, L. F. Fraceto, Polymeric nanoparticles as an alternative for application of gibberellic acid in sustainable agriculture: a field study, *Sci. Rep.* **9**, 1 (2019). <https://doi.org/10.1038/s41598-019-43494-y>
26. N. Verbruggen, C. Hermans, Proline accumulation in plants: A review, *Amino Acids*, **35**, 4 (2008). <https://doi.org/10.1007/s00726-008-0061-6>

27. P. E. Verslues, S. Sharma, Proline Metabolism and Its Implications for Plant-Environment Interaction, *Arabidopsis Book*, **8** (2010). <https://doi.org/10.1199/tab.0140>
28. S. Hayat, Q. Hayat, M. N. Alyemeni, A. S. Wani, J. Pichtel, A. Ahmad, Role of proline under changing environments: A review, *Plant Signaling and Behavior*, **7**, 11 (2012). <https://doi.org/10.4161/psb.21949>
29. A. Tanaka, R. Tanaka, Chlorophyll metabolism, *Curr. Opin. Plant. Biol.* **9**, 3 (2006). <https://doi.org/10.1016/j.pbi.2006.03.011>
30. R. Mattioli, P. Costantino, M. Trovato, Proline accumulation in plants: not only stress., *Plant signaling & behavior*, **4**, 11 (2009). <https://doi.org/10.4161/psb.4.11.9797>
31. Q. Wang, Y. Zhang, H. Hu, J. Hu, M. Xiang, Q. F. Yang, Comparative proteomics analysis of the responses to selenium in selenium-enriched alfalfa (*Medicago sativa* L.) leaves, *Plant Physiology and Biochemistry*, **165** (2021). <https://doi.org/10.1016/j.plaphy.2021.04.039>
32. P. J. White, Selenium metabolism in plants, *Biochimica et Biophysica Acta - General Subjects*, **1862**, 11 (2018). <https://doi.org/10.1016/j.bbagen.2018.05.006>
33. P. J. White, Selenium in soils and crops, in *Molecular and Integrative Toxicology*, Springer Science+Business Media B.V. (2018), [https://doi.org/10.1007/978-3-319-95390-8\\_2](https://doi.org/10.1007/978-3-319-95390-8_2)