Beneficial dose-dependent effects of Ag nanoparticles on germination do not compromise growth and metabolites of *Capsicum annuum* seedlings.

Berenice Cortes Espinoza[1], Alejandro Sánchez-González[2], Evarista Arellano-García[3], Rafael Bello-Bedoy[1]

[1] Facultad de Ciencias, Universidad Autónoma de Baja California, Ensenada, Baja California, México.
[2] Facultad de Ciencias, Universidad Autónoma de Baja California, Ensenada, Baja California, México.
[3] Facultad de Ciencias, Universidad Autónoma de Baja California, Ensenada, Baja California, México.

Corresponding author:

Rafael Bello-Bedoy

Ensenada, Baja California, México.

Email: address: rbello@uabc.edu.mx

Abstract

This study evaluates the effects of silver nanoparticles (AgNP) as a nanopriming and its potential detrimental impact on growth and physiology of a wild and domesticated variety of chili seeds (*Capsicum annuum*), comparing wild (Chiltepin) and domesticated (Serrano) varieties. Seeds were soaked for 24h in AgNP Argovit™ solutions of 0ppm, 50ppm, 100ppm, and 250ppm. Germinations were recorded for 14 days in replicated Petri dishes in a controlled growth chamber, and germination was recorded. A subsample of germinated plants from each of the four treatments was transplanted ~~to~~into plastic pots to measure growth and physiology at 28 and 42 days. On each day, three different plants were randomly chosen to measure shoot and root length and biomass. Our ~~physiology~~physiological measurements included primary and secondary metabolites, including chlorophyll and polyphenols, respectively. Additionally, potential genotoxic effects were investigated by analyzing meristematic tissue (root apical zone) exposed to a 5ppm nanoparticle solution for 72 hours. The results indicate that AgNPs increased the germination rate up to 90% in wild chili plants, compared to 77% in the control group, without adverse effects on plant development. No significant differences were observed in growth measurements, chlorophyll production, polyphenol content, or the number of dividing cells. Furthermore, no chromosomal aberrations were detected in the analyzed cells. Our findings showed that the beneficial effects of nanopriming did not affect wild or domesticated chili seedlings. Also, Serrano chili exhibited lower sensitivity to nanopriming, suggesting that domestication reduces its response to external factors such as nanoparticles. This study highlights the benefits of nanopriming with AgNPs and supports the safety of this technology in agriculture even at high concentrations.

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Keywords

Nanotechnology; agriculture; seeds germination; phytotoxicity; silver nanoparticles.

1. ~~INTRODUCCIÓN.~~INTRODUCTION

The process of germination is critical for adequate plant establishment to ensure conservation of wild populations and efficient production of resources in cultivated plants (Hay & Probert, 2013). This situation is particularly evident in high-value crops which include both wild and cultivated plants, where there is a demand for conserving genetic resources of the ancestors of cultivars to increase the production of cultivated varieties (Mastretta-Yanes et al., 2024)and to preserve biological diversity and culture (Tobón-Niedfeldt et al., 2022). The adoption of technologies to benefit wild plant conservation and crop yield are highly desirable. Nanoparticles are considered a technology to solve problems in agriculture in recent years (Mahakham et al., 2017), and those beneficial effects can be adopted on wild plants. However, the implementation of nanoparticles can expose plants to negative impacts that affect growth and reproduction (i.e., genotoxity, phyto-toxicity). Thus, to face the problem of low germination rates and establishment as a result of seedling sensitivity to nanoparticles stresses, it is needed to evaluate the potential of this technology.

Seed priming using nano-particles (i.e., nano-priming) is a pre-sowing treatment that employs nanoparticles of different materials in an aqueous solution (Nile et al., 2022). The application of nano-primming can induce changes by modifying physiological and biochemical processes and its expression, positively influencing germination (Mahakham et al., 2017), growth (Acharya et al., 2020) and even metabolite synthesis in different crops (Almutairi & Alharbi, 2015; Imtiaz et al., 2023). However, the effects of nano-priming have also shown detrimental effects on genetic material such as chromosomal aberration and nuclei breaks (ie., genotoxicity (Kumari, Mukherjee & Chandrasekaran, 2009; Patlolla et al., 2012) and plant growth such as root elongation (*i.e.,* phyto-toxicity) (Thuesombat et al., 2014; Almutairi & Alharbi, 2015; De Paiva Pinheiro et al., 2020). Thus, the negative impact ~~to~~on plant growth and

metabolites synthesis questions the efficacy of those nanoparticles. In particular, considering a possible scenario where nanoparticles dispersed in soil and water could become an environmental problem.

Nanoprimming effects with Silver nanoparticles (AgNPs) effects in plant biology are complex. Recent review of empirical evidence has shown that different effects on plants strongly depend on genetic differentiation and/or AgNP dosage (Imtiaz et al., 2023; Khan et al., 2023). For instance, the germination response of diploid and triploid water melon differed, finding an increased germination and early vegetative growth in a diploid cultivar exposed nanoprimming with silver particles, but its effects were absent on a triploid water melon variety (Acharya et al., 2020), indicating that silver particle depends on genetic background. Similarly, the concentration of silver nanoparticles used in nanoprimming treatments contributes to variability in results (Imtiaz et al., 2023). Different studies have been found effects of AgNPs nanoprimming are variable for germination and early growth and development in wheat, rice, watermelon, zucchini (Almutairi & Alharbi, 2015; Acharya et al., 2020; Santhoshkumar, Hima Parvathy & Soniya, 2024). Moreover, negative effects were found at higher concentrations for onion (Kumari, Mukherjee & Chandrasekaran, 2009) and wheat (Vannini et al., 2014). A general conclusion is that AgNPs' effects ~~of~~on seed nano-primming strongly depend on the cultivar and that the dose-effect also ~~depend~~depends on the cultivars~~.~~

One of the major sources of genetic differentiation is artificial selection during the ~~process of~~ domestication and genetic improvement processes (Mostert-O'Neill et al., 2022). The abrupt difference between varieties and wild progenitors modifies the response of plants to exogenous biotic and abiotic factors, because domestication reduces genetic diversity that is often accompanied with changes in germination and plant growth requirements and the synthesis of plant metabolites (Varela Milla et al., 2013). Given that domestication alter various responses

of plants, such as chemical defense (Shlichta et al., 2018), it is expected that wild and domesticate cultivars show a different response to the exposure of nanoparticles. Based upon this evidence, we expected to find variation in the response on germination and growth in wild and domesticate plants exposed to silver nanoparticles.

Mexican *Capsicum annuum* varieties entail a group of plants that currently show different levels of domestication (Aguilar-Meléndez et al., 2009), since the wild ancestor of chilies *Capsicum annuum* var. *glabriusculum* up to more than 60 that has been under selection for about 6400 years ~~bp~~BP (Araceli et al., 2009; Kraft et al., 2014). As a result, there drastic variation in seed size and germination rates that need attention to improve germination in wild and domesticate varieties (Granata et al., 2024). For instance, wild chili seeds of *Capsicum annuum* var. *glabriusculum* have low germination rates (Hernández-Verdugo et al., 2010; Cano-Vazquez et al., 2015), which has been related to its smaller in size and limited number of reserves to support germination and seedling growth (García Federico et al., 2010). In contrast, cultivars have a larger seed and have been under strong selection, implying that germination and seedling growth will be higher in cultivated varieties. Particularly for Mexican chili cultivars domesticated in Mexico, no information about germination rates and establishment has been reported (Hernández-Verdugo, Oyama & Vázquez-Yanes, 2001). In the case of wild chilies, there are several studies about germination, but no method has been found to consistently improve germination rates, despite various efforts. This absence of information is critical regarding natural and agricultural resources ~~of a~~that are in high demand.

This study evaluates the effects of silver nanoparticles (AgNPs) on germination, growth, and morphology in wild and domesticated chili plants, focusing on dose-dependent responses. We aim to identify optimal concentrations for growth while assessing phytotoxic and genotoxic risks, hypothesizing that wild chili benefits from AgNPs will be higher for wild plants that possess small seeds and ample variation in germination success and growth rates,

whereas larger seed reserve and high germination and growth rates of the domesticated cultivars will not increase. Genotoxic effects are further assessed via root mitotic index analysis. Operationally, we compare the effect of silver nanoparticles on domesticated and cultivated chili plants at three concentrations (50, 100, 250 ppm) (Sanchez-Perez et al., 2024) to understand the concentration that maximizes germination and growth of chilis, or if it provokes geno- and/or phyto- toxic response in terms of germination, mitotic index and seedling growth.

2. MATERIALS AND METHODS

Study specie

Capsicum annuum L. (Solanaceae) is a perennial and flowering shrub with multiple varieties. Plants usually measure less than one meter in height, their leaves have smooth margins, and their roots are branched and fibrous. Their fruits vary greatly in color, shape, size, and spiciness depending on the variety. The flowers are small and white sometimes with pink or purple undertones. There are wild (*Capsicum annuum* var. *glabriusculum*) and domesticated variations (*Capsicum annuum* var. *annuum*)

Seed germination trials

The germination trials were conducted using seeds of the domesticated cultivar chile serrano and its wild counterpart. Wild chili seeds were collected from their natural habitat in Sonora, ~~México~~Mexico, whereas commercial serrano was obtained at a commercial market. The seeds of the serrano variety (domesticated) had a mean seed weight of 4.16 (207.9mg/50 seeds) and wild chili seeds had a mean weight of 2.6mg (132.7 mg/50 seeds). Before germination assays, the seeds were washed with 70% ethanol for 2 minutes and then rinsed three times with distilled water. Seeds were placed in an eight cm diameter Petri dish with filter paper with 10 seeds per

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dish of each variety. Each test was replicated five times. The seeds were treated with 5 ml of each treatment. The four treatments applied were 0, 50, 100, and 250 ppm of silver nanoparticles Argovit™ (AgNPs). Each treatment had four replicates. The Petri dishes were sealed with Parafilm and placed in a controlled growth chamber set to a temperature range of 24-27°C. The chamber was programmed for a photoperiod of 16 hours of light followed by 8 hours of darkness, and the samples were incubated for 14 days.

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Germination percentage

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A seed was registered germinated, when the radicle was observed. We registered germination in a binomial fashion as follow: 1= germinated and 0= no germinated. Day to germination was registered since the day, there were treated. The number of germinated seeds was counted every day, and along 14 days of germination, where most seeds had germinated. Furthermore, we calculated Total germination percentage (%) and germination rate. The germination percentage (~~G%~~G %) was calculated as the ratio of germinated seeds to the total number of incubated seeds.

At germination, each seed was transplanted individually into trays of 50 cells filled with sterile commercial soil mixture (Berger BM2). When seedling had two leaves well developed, each seedling was individually transplanted into a 3.5 inches pot filled with the commercial mix-soil. Further, when seedlings had four leaves, seedlings were transplanted into 2.5 L pots for full growth. Wild and cultivated chilies produce roots abundantly; therefore, seedlings were transplanted twice during the experiment, ensuring proper root growth. Transplants were carried avoiding root damage to prevent any stress for the plant.

All plants were irrigated with 100 mL of water every other day taking care of maintaining soil moisture. Seedlings were fertilized weekly with 25 mL of an NPK 19-19-19 solution, 3g/L.

Measurement of morphological attributes

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To assess the effect of nano-primming on growth and development of wild and cultivated chili, the following measurements were taken at 28- and 42-days after germination: root and shoot length, fresh and dry biomass weight of the root and shoot, and height. The root length was measured using the main root as guideline, from the crown at the base of to the tip of the radicular tip. The shoot was measured by the base of the stem to the terminal apical bud of main stem.

Fresh biomass was obtained by carefully removing the root from the substrate and thoroughly cleaning it with distilled water, then drying it with blotting paper and weighing it on an electronic balance (Mettler Toledo). Finally, the biomass wrapped in aluminum foil was dehydrated in a VWR convection oven at 65°C for 7 days to ensure that seedlings were completely dehydrated. Further it was weighed on ~~ana~~ precision balance (Mettler Toledo MSQ205) to the nearest 0.0001 gr.

Quantification of total polyphenol content

Following the Folin-Ciocalteu method, the total phenolic content in fresh plant leaves was quantified at 28- and 42-days after germination. First, properly labeled Eppendorf tubes were weighed. Subsequently, the leaves were cleaned to remove any substrate residue. Once the sample was clean, it was macerated in an Eppendorf tube and weighed. It was then incubated in 1 mL of hydro-methanolic solution (methanol 100%) for 24 hours in the dark.

After 24 hours, it was centrifuged at 13,000 rpm for 5 minutes. In new Eppendorf tubes, 1 mL of distilled water, 20µL of extract from each sample, and 100µL of Folin-Ciocalteu phenol reagent (Sigma-Aldrich) were added and shaken. Afterward, 300µL of sodium carbonate ($NaCO_3$) solution was added and shaken. They were then incubated for 2 hours at room temperature. After 2 hours, the total amount of phenols in the samples was measured in

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a monochromatic spectrophotometer (Hach DR 2800) at λ=765 nm, using gallic acid as the standard (Ainsworth & Gillespie, 2007). Distilled water was used as a blank.

Chlorophyll Content Index

The Leaf Chlorophyll Content Index (CCI) was measured using the Chlorophyll Content Meter-200 plus (OPTI-SCIENCE, Hudson NH, USA). The meter determines the amount of chlorophyll through absorbance obtained in an area of 0.71 cm^2 at 600 and 900 nm (±1.0). Chlorophyll from the fourth fully developed true leaf was measured. An average of three measurements was calculated for each leaf per plant.

Cell division indicators

To examine the possible negative effects of nano-priming on cell division in apical zones, we exposed roots and shoots to an AgNP solution and use water as control. For this, we germinated wild and domesticated seeds. Once seedling had the third to fifth true leaves, 20 individuals of each variety were randomly chosen to receive the experimental treatment, five plants for each treatment. The plants were dug up, washed, and placed with their roots submerged in 150 ml beakers containing 120 ml of solution from each treatment. The plants were exposed to an AgNPs solution at 5 ppm. All seedlings were left in each treatment for 72 hours, with shoot and root growth measured every 24 hours. After 72 hours, the staining of the dividing cells in the roots was carried out.

Root and shoot preparation

The observation protocol for dividing cells modified by (Rohami et al., 2010) was then carried out. Briefly, horizontally growing lateral roots were cut from the apical zone at an average distance of 1 cm. The cuts of the roots were placed individually in 1.5 ml microtubes containing a solution of ethanol and glacial acetic acid (3:1) for 24 hours at room temperature.

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Subsequently, hydrolysis was performed using 1 N Hydrochloric Acid (HCl) for 30 minutes at room temperature on a watch glass. The roots were then individually stained on microscope slides with Aceto-Orcein (Sigma-Aldrich) for 30 minutes at room temperature. After staining, the excess was removed by immersing the roots in two washes of acetic acid. The squash technique was then applied on the microscope slide by adding a drop of acetic acid to the root. Gentle pressure was applied using a pencil eraser with light movements to spread the cells.

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They were observed under an optical microscope using a 100X objective with immersion oil. A total of 500 to 2000 cells per sample were counted to estimate the cell division (mitotic) index and determine the proportion of mitosis phases in the samples. The cell division index was assessed by counting the number of dividing cells relative to the total number of cells counted. Meanwhile, the phase index was calculated by counting the number of cells in a specific mitosis phase (prophase, metaphase, anaphase, and telophase) relative to the total number of cells in the sample.

Statistical analysis~~.~~

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To examine the effects of nano-priming on wild and cultivated chili, a logistic ANOVA of germination was conducted. The model included the fixed effects of chili type (wild ~~vs~~vs. cultivated), nano-priming treatment and the interaction type x variety. Growth and biochemical traits namely, fresh and dry biomass, root and shoot length, phenolic and chlorophyll concentration were measured by means of a two wan analysis of variance (Two-way ANOVA). The analyses included the fixed effects of type (Wild ~~vs~~vs. Domesticate), AgNPs concentration treatment and the plant type x treatment interaction. Variations in mitotic phases were analyzed by means of a two wan analysis of variance (Two-way ANOVA). The analyses included the fixed effects of type (wild, domesticate), AgNPs, urea and ammonium sulfate exposure

concentration treatments and the type x treatment interaction. All statistical analyses were performed using the JMP 10 (SAS).

3. RESULTS

Germination

The exposure to silver nanoparticles (AgNPs) significantly affected total seed germination, with significant differences observed between wild and domesticated genotypes (Table 1; Fig. 1). The control group showed 77% germination, but treatment with 100 ppm increased germination to 90%, and 50 ppm treatment achieved 82%. Moreover, the ANOVA detected a significant plant type × treatment interaction, indicating that dosage effect was different between wild and cultivated chilies. The only variety that was influenced by the concentration of AgNPs was the wild one (Fig. 1), with an increase in the germination percentage as the concentration of nanoparticles increased. Total germination of the wild variety exposed to 100 ppm and 250 ppm showed a higher germination in relation to its control treatments. In contrast, seeds in the silver nanoparticle treatments did not differ from the control treatment (Fig. 1). Germination rate of wild and cultivated chili were 86% and 83.5%, and they did not show statistical difference among them ($\chi^2 = 3.33$; P=0.06). Referring to the survival test, we only found significant differences within domesticated varieties, being different the control group respect to the 250 ppm nanoparticle treatment (Table S1). The wildtype genotype showed the same velocity rate in all the treatments (Table S1).

Growth and morphological attributes

We found that the only differences between varieties at 28 days of germination were in shoot and total length, total wet biomass (both root and shoot), dry shoot weight, and total dry biomass weight (Table S2). Regarding the effects of nanoparticles, no significant effects were found, nor in the interaction.

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In 42-day-old plants, significant treatment effects were observed only for the total wet mass (p = 0.04; Table S3). However, this effect was not dependent on the variety. The effect of nanoparticles (50, 100 and 250 ppm) in relation to the variety was significant only for the total length (p = 0.05; Table S3; Fig. S1).

Total polyphenol content

Total polyphenol levels showed no significant differences between plant varieties or AgNPs treatments at 28 and 42 days after germination (Table S4). There were also no differences in the interaction between the type of plant and the treatment (p=0.5). However, it can be noted that in both measurements of the Serrano variety (domesticated) there is an increase in the polyphenol content starting from the highest concentrations of nanoparticles (100 and 250 ppm) (Fig. S2). In the case of Chiltepin (wild) there are irregularities in the quantity of polyphenols recorded in both measurements. The lack of a consistent effect across varieties and time points may reflect differences in the plants' metabolic priorities during different developmental stages.

Chlorophyll content

Chlorophyll content differed significantly between varieties 28 and 42 days after germination (Table S5). At 42 days, the effect of AgNPs was variety-dependent, with a reduction in chlorophyll content observed as nanoparticle concentration increased in the domesticated varieties (Fig. S3).

Cell division indicators: Phytotoxic and Genotoxic evaluation

Evaluation of stem and root length during the first 72 hours of exposure revealed significant differences between varieties in all measurements ($p < 0.0001$) (Table S6). Regarding the growth (cm) of the shoot and root of the plants, there were no effects of the treatment in any of

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the varieties. Although the analyses of the leaf area of the plants at 48 and 72 hours in the interaction of the variety with the treatment were significant in the ANOVA (Table S6), the Tukey test reflects that the differences are between the varieties and not in the treatments. This shows that there are no differences between the treatments in the varieties (Table S7). These results suggest that AgNPs did not exhibit any phytotoxic effects under the conditions tested.

In the genotoxic evaluation, no significant effects on the mitotic index or chromosomal aberrations in root cells were observed for any variety (Table S8). In wild chili cells, treatment with nanoparticles increased the number of dividing cells compared to the control, while in domesticated varieties, a decrease in all phases of mitosis was observed with AgNPs treatments. This differential response may be attributed to genetic differences between the wild and domesticated genotypes.

4. DISCUSSION

This study investigated the effects of nanopriming with silver nanoparticles (AgNPs) at various concentrations on the germination and early growth of both wild and domesticated varieties of *Capsicum annuum*. It evaluated primary and secondary metabolism, including total phenol and chlorophyll content. The results indicated that higher concentrations of AgNPs improved germination rates in wild seeds, while no significant effects were observed in domesticated seeds. Furthermore, the study found no negative changes in leaf biochemistry, nor were there any cytotoxic or genotoxic effects on meristematic tissues. These findings suggest that nanoprimming with AgNPs may enhance germination without adversely affecting the early development of these *C. annuum* varieties (Mahakham et al., 2017; Shelar et al., 2021).

Variability in response to AgNPs between wild and domesticated varieties

The effects of silver nanoparticles vary greatly between studies as a function of plant species and concentrations (Mays et al., 2024), but the natural history and the history of domestication

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of cultivars has not been considered in spite of numerous studies indicating that domestication profoundly modifies genotypes and phenotypes and their functional responses(Varela Milla et al., 2013; Serrano-Mejía et al., 2022). Our results in a wild and a domesticated *C. annumm* suggest that differences in seed biology are a relevant factor in the response to AgNPs treatments. In this case, wild *C. annuum* seeds exposed to nano–primming at high doses (100ppm and 250 ppm) increased germination~~s~~ rates ~~indication~~indicating that its beneficial effects on this variety are ~~does~~dose dependent, in comparison to the domesticated cultivar that showed no response at any of the treatments. This suggests that differences in seed biology influence the response to nanopriming, like results found in maize (Thongmak et al., 2022).

In relation to wild seeds, nano–primming can favor germination by promoting physiological process such as imbibition and hormonal functioning. The absorption of AgNPs by the seeds, causing modifications in physiological responses related to the activation of in phytohormones implicated in growth and in the break of dormancy (Méndez-Argüello et al., 2016) and/or by the activation of genes related to the cell proliferation (Qian et al., 2013). For instance, nano-priming can enhance the activity of the α-amylase, which is a factor critical for seed germination (Mahakham et al., 2017). The low germination rates of wild chilies have been treated by exposing seeds to variable concentration of Gibberelic acid (GA_3, which is limited under natural conditions) (Hernández-Verdugo, Oyama & Vázquez-Yanes, 2001). Despite the variability of results, most have shown that the exposure to exogenous gibberellins increase germination rates, suggesting that germination in wild chilies is limited by this hormone (Hernández-Verdugo, Oyama & Vázquez-Yanes, 2001; Cano-Vazquez et al., 2015). Thus, nanoparticles could favor water absorption and the activation of phytohormones relevant for seed germination even under limited concentrations. A study on *Vanilla planifolia* indicates that safe concentrations of silver nanoparticles (AgNPs), specifically 25 and 50 mg/L, can promote growth without causing significant genotoxic effects.

However, prolonged exposure to higher concentrations may lead to chromosomal abnormalities (Casillas-Figueroa et al., 2020). This underscores the importance of carefully adjusting doses according to the biological context (Spinoso-Castillo et al., 2017; Bello-Bello et al., 2018).

A raising question is why the domesticated species was not influenced by AgNP nano primming. Two factors could account for the absence of response to the nano-priming in these plants. The first ~~one~~ is that domestication limited the response of chili seeds to nanoparticles. However, we considered that this is not a plausible explanation, because previous studies in *Capsicum annuum* have found that seeds and seedling show variation in germination and growth in response to the exposure to different AgNPs (Yuan et al., 2018; Sánchez-Pérez et al., 2023). A plausible explanation is that seeds of domesticate *C. annuum* have evolved an efficient response to hydropriming that activates gibberellic acid and cytokines. Thus, the addition of AgNPs that improve germination in wild chilies did not increased germination above hydropriming in domesticate plants. Further research is needed to understand how domestication has shaped germination mechanisms and their relationship to nanoprimming technology (Song & He, 2021; Thongmak et al., 2022).

Evaluation of the cytotoxic and genotoxic impact of AgNPs

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An important objective of this study was to determine whether exposure to AgNPs generated cytotoxic or genotoxic effects in meristematic tissues of wild and domesticated varieties of *C. annuum.* The results indicated that the nanoparticles adversely affected neither germination nor initial plant growth. Evaluation of mitotic index and chromosomal aberrations confirmed the absence of genotoxic effects in both varieties. These results are consistent with studies reporting the absence of phytotoxicity of silver nanoparticles at low to moderate concentrations (Budhani et al., 2019). However, it is important to note that higher concentrations can induce

adverse effects, as observed in other plant systems (Kumari, Mukherjee & Chandrasekaran, 2009). In *Allium cepa*, for example, concentrations up to 100 µg/mL of Argovit™ were shown to promote growth without genotoxic or cytotoxic damage (Casillas-Figueroa et al., 2020). Our study found that primed and non-primed seedlings had similar growth rate, attained a similar size and had accumulated similar amounts of foliar chlorophyll and phenolic content 28-days after germination (Table S1). Indeed, a further assay found no negative impact of AgNps on apical or radicular meristem, supporting the notion that AgNPs ~~they~~ did not have a phytotoxic effect on the ability of plants to grow. This study's tolerance of wild and domesticated varieties to concentrations up to 250 ppm suggests that these nanoparticles could be a safe tool for improving germination and growth in selected crops (Acharya et al., 2020; Thongmak et al., 2022; Granata et al., 2024).

An obvious discussion of ~~this~~these results is why silver nanoparticles did not provoke an alteration ~~of~~in growth or metabolism in *C. annuum*. The effects of silver nanoparticles in plants are diverse in research, with some indicating phytotoxicity, cause inhibition in germination and growth, stress in plants by manipulating oxidative stress or osmotic stress action (Budhani et al., 2019), while others promote growth, germination, or even act as nano-pesticides and nano-fertilizers (Khan et al., 2023). Our findings agree with those studies demonstrating that (Argovit™) silver nanoparticles do not induce phytotoxic effects at low concentration ranges (5–50 mg + L) while concentrations above may exhibit inhibitory effects, as observed in *Gerbera jamesonii*. The absence of ~~-~~phytotoxic effects at higher concentrations on suggest that both wild and domesticated plants tolerate high levels of exposure to silver nanoparticles.

Implications and future of ~~n~~Nanoprimming in ~~a~~Agriculture

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Our findings underscore the importance of customized approaches in agricultural research. AgNPs significantly impact plants genetic and physiological context, and this study demonstrates the need for tailored solutions to maximize their benefits (Thuesombat et al., 2014; Thongmak et al., 2022). This is particularly important in a context where sustainable agriculture requires innovative technologies. AgNPs could offer a viable solution to overcome germination and early plant establishment challenges, especially in varieties with limited germination rates (Song & He, 2021; Singh et al., 2023). Additionally, while this study indicates that AgNPs may be a viable option to address germination challenges in wild plants, their effects on other *C. annuum* varieties and cultivars of other species require further investigation to understand whether AgNPs are useful in Capsicum. Otherwise, our knowledge of exposure to AgNPs will be remain limited to each particular experiment.

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Future research should focus on understanding the physiological and molecular mechanisms underlying the interaction between nanoparticles and seeds (Mahakham et al., 2017). In addition, it is essential to assess the long-term impact of AgNPs on the environment and human health and explore their application in the broader range of agricultural species and conditions (Santhoshkumar, Hima Parvathy & Soniya, 2024). This will help define safe and sustainable limits for the use of~~using~~ nanotechnology in agricultural systems.

5. CONCLUSION

This study demonstrates the benefits of ~~n~~Nanopriming using silver nanoparticles (AgNPs) in germinating wild *Capsicum annuum* seeds. Importantly, it confirms the safety of this technology, as no adverse effects on phytotoxicity or genotoxicity were observed (Casillas-Figueroa et al., 2020). Although domesticated seeds did not show significant responses, the findings highlight the potential of this technology to improve germination in wild varieties without negatively impacting early growth or metabolic processes.

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Tables

Table 1. Estimates of the logistic regression of germination success as a function of *Capsicum annuum* variety (wild vs cultivated), treatment of silver nanoparticles exposure, and its interaction.

Source of variation	***d.f.***	X_2 ***L-R***	***P***

Plant type	1	3.33	0.0679
Treatment (Ag ppm)	3	12.13	**0.0069**
Plant type × Treatment (Ag ppm)	3	8.68	**0.0338**

Table S1. Survival test for germination rates of *Capsicum annuum* domesticated (DM) and wildtype (WT) varieties after 14 days of treatment. Chi-square test.

Plant type	**Test**	**Chi square**	***d.f.***	***P***
Domesticated	Log-Rank	11.35	3	**0.01**
	Wilcoxon	20.22	3	**0.0002**
Wildtype	Log-Rank	2.65	3	0.449
	Wilcoxon	1.02	3	0.7975

Table S2. Estimates of ANOVA of morphological traits of 28-days after germination plants, as a function of *Capsicum annuum* variety (wild vs cultivated), treatment of silver nanoparticles exposure, and its interaction.

Trait	**Source**	***d.f.***	**ss**	**F ratio**	***P***
Shoot length (cm)	Plant type	1	3.42	6.57	**0.02**
	Treatment (Ag ppm)	3	2.90	1.86	0.16
	Plant type × Treatment (Ag ppm)	3	4.30	2.76	0.06
Root length (cm)	Plant type	1	34.04	2.47	0.13
	Treatment (Ag ppm)	3	33.85	0.82	0.49
	Plant type × Treatment (Ag ppm)	3	48.73	1.18	0.33
Total length (cm)	Plant type	1	59.05	4.06	**0.05**
	Treatment (Ag ppm)	3	51.67	1.18	0.33
	Plant type × Treatment (Ag ppm)	3	81.51	1.87	0.16
Shoot wet mass (g)	Plant type	1	0.03	25.52	**<0.0001**
	Treatment (Ag ppm)	3	0.00	0.65	0.59
	Plant type × Treatment (Ag ppm)	3	0.00	1.13	0.35

Root wet mass (g)	Plant type	1	0.03	5.63	**0.02**
	Treatment (Ag ppm)	3	0.03	2.32	0.09
	Plant type × Treatment (Ag ppm)	3	0.01	0.75	0.53
Total wet mass (g)	Plant type	1	0.11	11.78	**<0.0001**
	Treatment (Ag ppm)	3	0.03	1.07	0.37
	Plant type × Treatment (Ag ppm)	3	0.01	0.37	0.77
Shoot dry mass (g)	Plant type	1	0.00	11.44	**<0.0001**
	Treatment (Ag ppm)	3	0.00	0.86	0.47
	Plant type × Treatment (Ag ppm)	3	0.00	2.19	0.11
Root dry mass (g)	Plant type	1	0.00	2.16	0.15
	Treatment (Ag ppm)	3	0.00	0.73	0.54
	Plant type × Treatment (Ag ppm)	3	0.00	0.64	0.59
Total dry mass (g)	Plant type	1	0.00	4.66	**0.04**
	Treatment (Ag ppm)	3	0.00	0.80	0.50
	Plant type × Treatment (Ag ppm)	3	0.00	0.81	0.50

Table S3. Estimates of ANOVA of morphological traits of 42 days after germination plants, as a function of *Capsicum annuum* variety (wild vs cultivated), treatment of silver nanoparticles exposure, and its interaction.

Trait	**Source**	***d.f.***	**ss**	**F ratio**	***P***
Root length (cm)	Plant type	1	23.26	3.67	0.06
	Treatment (Ag ppm)	3	4.75	0.25	0.86
	Plant type × Treatment (Ag ppm)	3	48.12	2.53	0.07
Shoot length (cm)	Plant type	1	50.40	202.21	**<0.0001**
	Treatment (Ag ppm)	3	0.60	0.80	0.50
	Plant type × Treatment (Ag ppm)	3	0.93	1.25	0.31
Total length (cm)	Plant type	1	142.13	19.99	**<0.0001**

	Treatment (Ag ppm)	3	4.39	0.21	0.89
	Plant type × Treatment (Ag ppm)	3	62.37	2.92	**0.05**
Shoot wet mass (g)	Plant type	1	0.10	8.72	**<0.0001**
	Treatment (Ag ppm)	3	0.06	1.79	0.17
	Plant type × Treatment (Ag ppm)	3	0.04	1.24	0.31
Root wet mass (g)	Plant type	1	0.02	1.80	0.19
	Treatment (Ag ppm)	3	0.12	4.22	**0.01**
	Plant type × Treatment (Ag ppm)	3	0.04	1.30	0.29
Total wet mass (g)	Plant type	1	0.20	5.30	**0.03**
	Treatment (Ag ppm)	3	0.36	3.16	**0.04**
	Plant type × Treatment (Ag ppm)	3	0.15	1.32	0.29
Shoot dry mass (g)	Plant type	1	0.00	3.24	0.08
	Treatment (Ag ppm)	3	0.00	2.37	0.09
	Plant type × Treatment (Ag ppm)	3	0.00	1.06	0.38
Root dry mass (g)	Plant type	1	0.00	4.13	**0.05**
	Treatment (Ag ppm)	3	0.00	2.17	0.11
	Plant type × Treatment (Ag ppm)	3	0.00	0.80	0.50
Total dry mass (g)	Plant type	1	0.00	3.62	0.07
	Treatment (Ag ppm)	3	0.00	2.37	0.09
	Plant type × Treatment (Ag ppm)	3	0.00	1.02	0.40

Table S4. ANOVA of total polyphenolic content on leaves of 28- and 42-days after germination plants, as a function of *C. annuum* variety (wild vs cultivated), treatment of silver nanoparticles exposure, and its interaction.

Trait	**Source**	***d.f.***	**ss**	**F ratio**	***P***
TPC (mg/g) 28-days	Plant type	1	0.69	1.10	0.30
	Treatment (Ag ppm)	3	1.59	0.85	0.48
	Plant type × Treatment (Ag ppm)	3	1.21	0.64	0.59

TPC (mg/g) 42-days	Plant type	1	0.00	0.00	0.96
	Treatment (Ag ppm)	3	2.91	0.98	0.42
	Plant type × Treatment (Ag ppm)	3	2.19	0.73	0.54

Table S5. ANOVA of Chlorophyll content on leaves measured after 28 and 42 days after germination, as a function of *Capsicum annuum* variety (wild vs cultivated), treatment of silver nanoparticles exposure, and its interaction.

Trait	Source	*d.f.*	ss	F ratio	*P*
ICC 28-days	Plant type	1	113.63	6.20	**0.02**
	Treatment (Ag ppm)	3	26.79	0.49	0.69
	Plant type × Treatment (Ag ppm)	3	50.48	0.92	0.45
ICC 42-days	Plant type	1	1250.42	84.47	**<.0001**
	Treatment (Ag ppm)	3	85.56	1.93	0.15
	Plant type × Treatment (Ag ppm)	3	246.49	5.55	**0.005**

Table S6. ANOVA results of root and shoot length of *C. annuum* at the start of exposure to AgNPs at 24, 48 and 72 hours.

Trait	Source	Estimate	Std error	t ratio	*p*
Root 0 h	Plant type	3.59	0.92	3.91	**0.0012**
	Treatment (Ag ppm)	-0.62	0.92	-0.67	0.5103
	Plant type × Treatment (Ag ppm)	0.73	0.92	0.8	0.4377
Root 24h	Plant type	3.83	0.92	4.16	**0.0007**
	Treatment (Ag ppm)	-0.72	0.92	-0.78	0.445
	Plant type × Treatment (Ag ppm)	0.59	0.92	0.64	0.5314
Root 48h	Plant type	4.02	0.93	4.31	**0.0005**
	Treatment (Ag ppm)	-0.88	0.93	-0.94	0.3616
	Plant type × Treatment (Ag ppm)	0.47	0.93	0.5	0.6218
Root 72h	Plant type	4.23	0.92	4.58	**0.0003**
	Treatment (Ag ppm)	-0.91	0.92	-0.98	0.3416
	Plant type × Treatment (Ag ppm)	0.50	0.92	0.55	0.5923
Shoot 0 h	Plant type	1.55	0.10	15.49	**<.0001**
	Treatment (Ag ppm)	0.15	0.10	1.45	0.1656

	Plant type × Treatment (Ag ppm)	0.19	0.10	1.87	0.0805
Shoot 24h	Plant type	1.65	0.10	16.1	**<.0001**
	Treatment (Ag ppm)	0.12	0.10	1.21	0.2457
	Plant type × Treatment (Ag ppm)	0.18	0.10	1.77	0.0964
Shoot 48h	Plant type	1.65	0.09	19.2	**<.0001**
	Treatment (Ag ppm)	0.04	0.09	0.41	0.687
	Plant type × Treatment (Ag ppm)	0.21	0.09	2.5	**0.0238**
Shoot 72h	Plant type	1.72	0.09	18.94	**<.0001**
	Treatment (Ag ppm)	0.10	0.09	1.09	0.2933
	Plant type × Treatment (Ag ppm)	0.25	0.09	2.79	**0.0131**

Table S7. LS Means Differences Tukey HSD of the interaction between the plant type and treatment of the shoot length of the plants after 48 and 72 hours of exposure to AgNPs treatment.

Trait	Level	Least Sq Mean	Std error
Shoot 48 h	Serrano,AgNPs	8.1394	0.17
	Serrano,dH2O	7.64	0.17
	SPCs,AgNPs	4.4126	0.17
	SPCs,dH2O	4.771	0.17
Shoot 72h	Serrano,AgNPs	8.5414	0.18
	Serrano,dH2O	7.8368	0.18
	SPCs,AgNPs	4.5926	0.18
	SPCs,dH2O	4.9022	0.18

Table S8. ANOVA results of the number of cells found in a certain phase of mitosis in roots of wild and domesticated *C. annuum* plants after 72 hours of exposure to a solution of silver nanoparticles (AgNP).

Trait	Source	Estimate	Std error	t ratio	*p*
Prophase	Plant type	0.00	0.00	2.34	**0.032**
	Treatment (Ag ppm)	0.00	0.00	-0.39	0.705
	Plant type × Treatment (Ag ppm)	0.00	0.00	-1.59	0.132
Metaphase	Plant type	0.00	0.00	1.74	0.101
	Treatment (Ag ppm)	0.00	0.00	-0.74	0.470

	Plant type × Treatment (Ag ppm)	0.00	0.00	-1.87	0.080
Anaphase	Plant type	0.00	0.00	-0.71	0.489
	Treatment (Ag ppm)	0.00	0.00	-0.61	0.553
	Plant type × Treatment (Ag ppm)	0.00	0.00	0.84	0.414
Telophase	Plant type	0.00	0.00	0.63	0.535
	Treatment (Ag ppm)	0.00	0.00	-1.39	0.185
	Plant type × Treatment (Ag ppm)	0.00	0.00	-1.32	0.206
Interphase	Plant type	0.00	0.00	-1.17	0.261
	Treatment (Ag ppm)	0.00	0.00	1.06	0.306
	Plant type × Treatment (Ag ppm)	0.00	0.00	1.19	0.253

Figures.

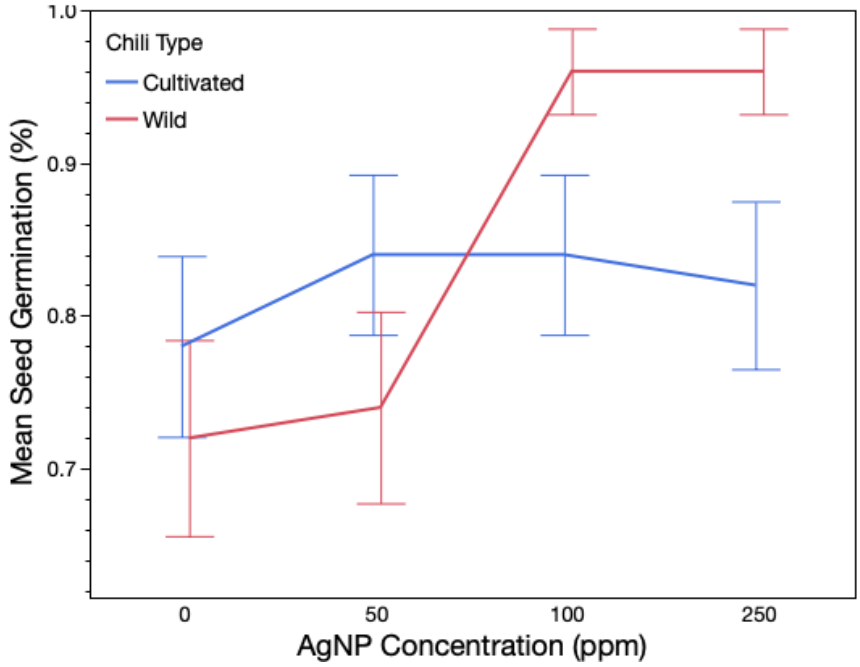

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Figure 1. Total germination of wild and domesticated *Capsicum annuum* plants exposed to three different doses of Ag nanoparticles. The figure shows mean germination and confidence intervals obtained from a logistic ANOVA. Red line refers to wild genotype (Chiltepin); blue line refers to domesticated genotype (Serrano).

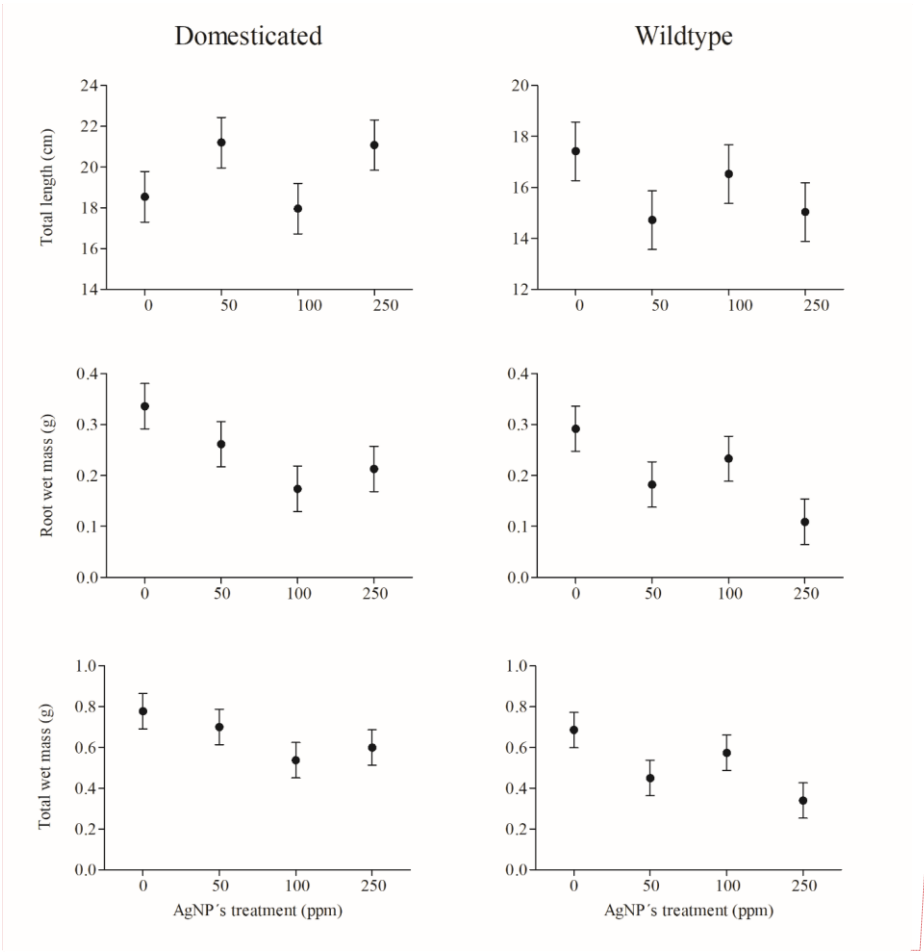

Figure 2. Total wet mass (g), Root wet mass (g) and Total length of domesticated and wild *C. annuum* plants after 42 days of germination at different AgNP concentrations (50, 100 and 250 ppm).

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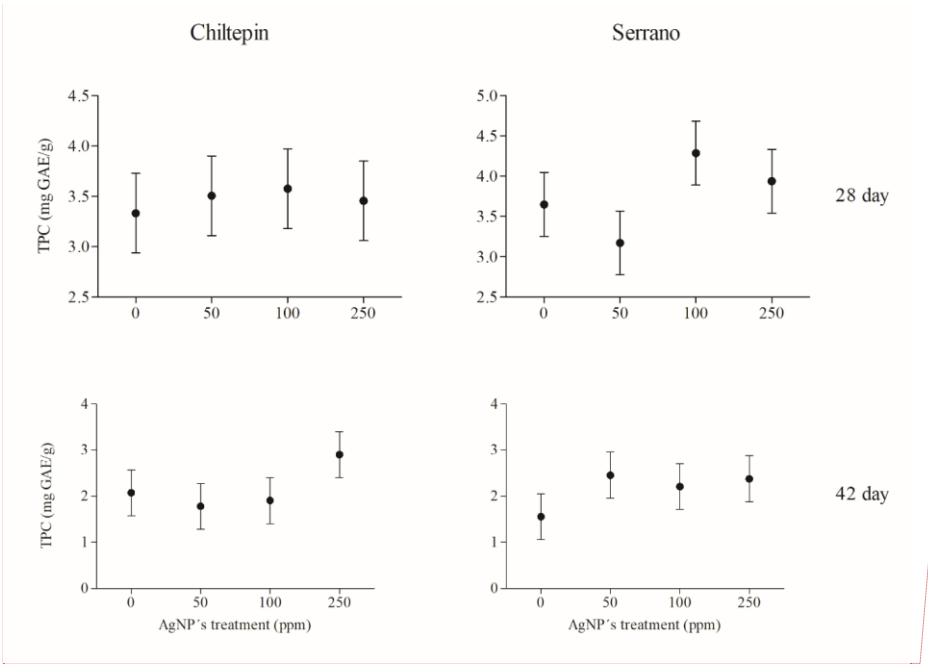

Figure 3. Total polyphenol content (TPC) of 28- and 42-days post-germination wild and domesticated plants with each silver nanoparticle (AgNPs) treatment (50, 100 and 250 ppm).

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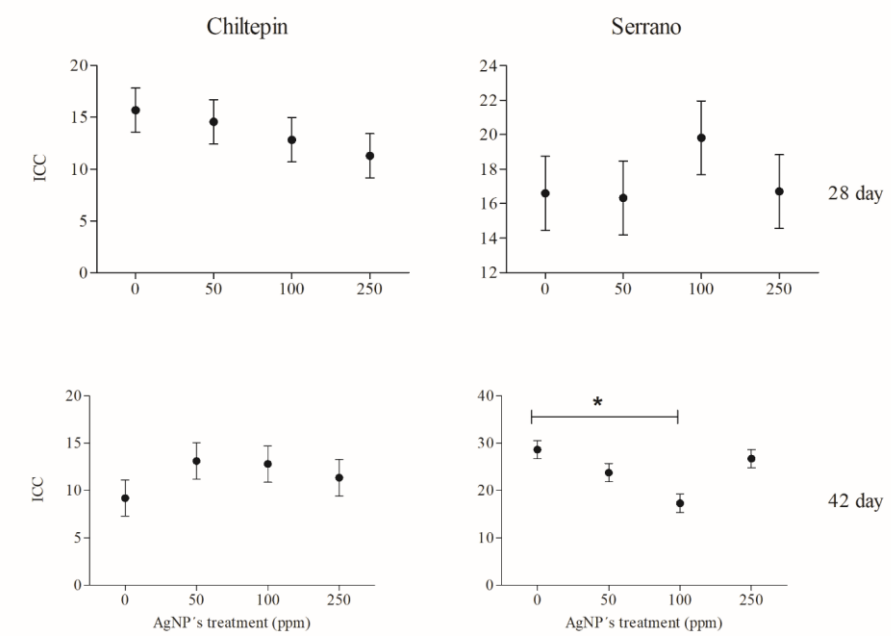

Figure 4. Chlorophyll content in leaves of 28- and 42-days post-germination wild (Chiltepin) and domesticated (Serrano) plants with each AgNP treatment (50, 100 and 250 ppm).