Kurukod Journal of Agriculture and Technology
Vol. 2 (1): 1-13 (November 2025)
ISSN: 2815-2298
DOI: 10.63798/kjat.v2i1/SNIIVGGaPAoCCaL

REGULAR ARTICLE

Silver Nanoparticle Improves *In Vitro* Germination, Growth and Pigment Accumulation of Coffee (*Coffea arabica* L.)

ARTICLE HISTORY

Received 29 September 2025
Revised 18 October 2025
Accepted 23 October 2025

Aron Louie L. Paler[1,2]*
ORCID: https://orcid.org/0000-0003-3837-9231

Catherine C. Arradaza[2]
ORCID: https://orcid.org/0000-0003-3181-3074

[1]School of Agriculture, Forestry and Environmental Studies, J.H. Cerilles State College, Mati, San Miguel, Zamboanga del Sur, Philippines
[2]Department of Horticulture, Faculty of Agriculture and Food Science, Visayas State University, Baybay City, Leyte, Philippines

*Corresponding author: aronlouie.paler@jhcsc.edu.ph

Abstract: This study investigated the effects of silver nanoparticles (AgNPs) on the *in vitro* germination, growth, and pigment accumulation of *Coffea arabica* L. zygotic embryos. Embryos were cultured on Murashige and Skoog (MS) medium supplemented with AgNPs at concentrations of 0.1, 0.5, 1.0, and 1.5 mg L^{-1}, arranged in a Randomized Complete Block Design with three replications. Results showed that AgNPs significantly improved the coefficient of velocity of germination and reduced mean germination time, with the most consistent effect observed at 0.5 mg L^{-1}. Morphological traits were also enhanced, with 1.0 mg L^{-1} of AgNPs producing the longest shoots and 0.5 mg L^{-1} of AgNPs inducing the longest roots and the earliest formation of true leaves. Pigment analysis revealed increased chlorophyll and carotenoid content under AgNPs supplementation, indicating enhanced photosynthetic capacity. The findings demonstrate that AgNPs, particularly at 0.5–1.0 mg L^{-1}, can accelerate germination, promote early growth, and enhance pigment accumulation in Arabica coffee embryos under *in vitro* conditions.

Keywords: arabica coffee; embryo culture; *in vitro* germination; silver nanoparticles; zygotic embryos

1. Introduction

Coffee is among the most valuable commodities in global trade. It has four main commercially cultivated species: Robusta, Liberica, Excelsa, and Arabica. However, Arabica is prized for its superior flavor and quality, accounting for 93.87 million bags in global coffee production (International Coffee Organization, 2022). Arabica is the sole self-fertile species within the Rubiaceae family (Gómez et al., 2023). In 2018, Arabica coffee accounted for over 23% of the Philippines' total coffee production, primarily cultivated in the country's highland areas (Philippine Statistics Authority, 2018).

Coffee production in the Philippines has declined over the past decade, despite its significant economic impact. Production decreased from 72,342 MT DC (36,171 MT GCB) to 60,640.95 MT DC (30,320.47 MT GCB) between 2015 and 2020, while yields decreased from 0.64 MT ha^{-1} to 0.54 MT ha^{-1} (Department of Agriculture, 2021). From 2019 to 2020, coffee production decreased by 4.2% worldwide (ICO, 2022). The same report noted that the country is only 15% self-sufficient in coffee, with imports accounting for about 81% of domestic consumption. Several factors, including uneven and slow seed germination and a lack of high-quality planting materials, cause this decline. These challenges show the pressing need for innovative strategies and the application of cutting-edge technologies to meet the growing demand both domestically and internationally.

The propagation of Arabica coffee presents notable challenges. Although seeds are the primary propagation method, this approach often results in uneven plantation establishment (Pinto et al., 2018). Coffee seeds are also known to germinate slowly, further complicating propagation. Farmers in Bansalan, Davao del Sur, and Pangantucan, Bukidnon, in the Philippines, reported that they are using seeds to propagate Arabica coffee. However, seed germination in the field is slow and uneven, which could be due to the inherent dormancy period of the seeds. Arabica seeds generally germinate in the following order: a) 32 days after harvest, b) 50 days after 8 weeks of storage, and c) 42 to 70 days after harvest (Wintgens & Zamarripa, 2004). *In vitro* propagation, also known as micropropagation, offers a more efficient alternative, enabling the rapid multiplication of high-quality planting materials under controlled, aseptic conditions.

In recent years, nanotechnology has emerged as a promising tool in plant tissue culture. Nanoparticles (NPs), particles smaller than 100 nm, possess unique physicochemical properties influencing plant growth, morphogenesis, pigment accumulation, and secondary metabolite production (Prasad et al., 2024). Among them, silver nanoparticles (AgNPs) are widely studied due to their antimicrobial properties, ability to modulate plant hormonal balance, and influence physiological and biochemical processes. Several studies demonstrate their significant potential in enhancing *in vitro* growth and development when applied at optimal concentrations. For instance, Tamimi and Othman (2023) reported that supplementing culture media with AgNPs improves shoot, root, and photosynthetic pigment accumulation in *Musa acuminata*. Similarly, Şener and Sayğı (2023) found that 0.1 mg L^{-1} AgNPs significantly promoted the growth and development of boysenberry under PEG-induced drought stress, demonstrating their ability to alleviate abiotic stress effects. Furthermore, Tung et al. (2021) revealed that adding 0.5 mg L^{-1} AgNPs to MS medium markedly improved *in vitro* growth, shoot multiplication, and plantlet survival in strawberry micropropagation, while reducing ethylene accumulation. However, the effects of AgNPs are not universal. Sami et al. (2020) highlight that silver nanoparticles' efficacy is dose-dependent and species-specific.

To the best of our knowledge, no published studies have investigated the effects of AgNPs on *Coffea arabica* zygotic embryo culture. Therefore, this study aims to examine the influence of AgNPs on *in vitro* germination, early growth, and pigment accumulation of arabica coffee. A treatment range of 0.1, 0.5, 1.0, and 1.5 mg L^{-1} was selected to capture both the stimulatory and potential inhibitory effects of AgNPs. By integrating nanotechnology into tissue culture, it aims to establish scientific data for nanotechnology-assisted micropropagation that can enhance the production of high-quality and uniform planting materials, thereby advancing plant biotechnology and supporting sustainable coffee production for the benefit of the farmers and the coffee industry.

2. Materials and Methods

2.1 Explant Collection

Arabica coffee (*Coffea arabica* L. var. Catimor) berries were collected from a reputable farm at Sitio Balutakay, Bansalan, Davao del Sur. The berries were carefully selected for size, maturity, and freedom from visible defects and disease to ensure uniformity. Immediately after harvest, the berries were

packed in jute sacks and transported to the Plant Tissue Culture Laboratory, Department of Horticulture, Visayas State University, Baybay City, Leyte, Philippines.

Upon arrival, one thousand (1000) berries were soaked in tap water, and floating fruits were discarded. The remaining berries were depulped to obtain seeds, which were soaked in tap water containing 10% fungicide (Dithane) for 12 hours. Mucilage was removed by rubbing seeds against each other under running water, and floating seeds were again discarded.

2.2 Surface Sterilization of Seeds

Seed disinfection was carried out under aseptic conditions. The seeds were first immersed in 95% ethanol for 5 minutes with continuous shaking, followed by two rinses with sterile distilled water. They were then surface-sterilized in 10% sodium hypochlorite (NaOCl) solution for 10 minutes, rinsed three times with sterile distilled water, and finally treated with 80% ethanol for 20 minutes inside the laminar flow hood. After three additional rinses with sterile distilled water, the sterilized seeds were prepared for zygotic embryo extraction.

2.3 Culture Medium Preparation

The medium used was Murashige and Skoog (1962) formulation supplemented with vitamins, Fe-EDTA, 30 g L^{-1} sucrose, and 5 g L^{-1} agar for solidification. The pH of the medium was adjusted to 5.8 ± 0.1 with either 1.0N HCl or 1.0N NaOH before dispensing. Culture vessels containing 15 mL of the medium were sterilized by autoclaving at 121 °C and 15 psi for 20 minutes (Murashige & Skoog, 1962).

2.4 Preparation of Nanoparticle Stock Solution

Silver nanoparticles (AgNPs) were procured from Guangzhou Hongwu Materials Technology Co., Ltd. (Guangzhou, China). Based on the Certificate of Analysis, the AgNPs had the following specifications: particle size 80–100 nm, surface area 4–8 m^2/g, tap density 1–2.5 g/mL, true density 10.5 g/cm^3, and purity 99.99%.

A stock solution of 100 mg L^{-1} was prepared following the modified protocol of Faraji and Sepehri (2019). Ten (10) mg of AgNPs were suspended in 100 mL of deionized water and sonicated (100 W, 40 kHz) for 30 minutes to ensure uniform dispersion. Working concentrations were prepared by serial dilution using the formula:

$$C_1V_1 = C_2V_2 \quad (1)$$

where C_1 is the concentration of the stock solution, V_1 is the volume of the stock solution required, C_2 is the desired concentration, and V_2 is the final volume of the working solution. Suspensions were vortexed before use to minimize aggregation, sterilized, and immediately incorporated into the culture medium.

2.5 Zygotic E*mbryo Extraction*

Under aseptic conditions, zygotic embryos were excised from sterilized seeds by carefully cutting open the endosperm with a sterile razor blade. Excised embryos were transferred individually into culture vessels containing 15 mL of medium corresponding to each treatment. The cultures were incubated at 25 ± 2 °C under cool white fluorescent light at 2,500 lux with an 8 h light/16 h dark photoperiod for four (4) weeks. Germination was monitored daily, using the emergence of cotyledons as the criterion for successful germination.

2.6 Plantlet Establishment

After the initial 4-week germination period, the germinated zygotic embryos were subcultured into fresh MS medium supplemented with vitamins, Fe-EDTA, 30 g L^{-1} sucrose, 2.0 mg L^{-1} BAP, and 5 g L^{-1}

agar. Two consecutive passages were conducted at 4-week intervals under the same growth conditions for plantlet establishment.

2.7 Data Gathered

2.7.1 Germination response

The germination of coffee embryos was assessed using the final germination percentage (FGP), coefficient of velocity of germination (CVG), and mean germination time (MGT). FGP represents the proportion of embryos that successfully germinated, CVG indicates the speed of germination over time, and MGT reflects the average duration required for embryos to germinate throughout the observation period.

2.7.2 Morphological characteristics

Morphological characteristics of coffee plantlets were evaluated based on shoot length, root length, the average number of true leaves, days to the formation of the first true leaf, and vigor index (VI). Shoot and root lengths were measured in millimeters from the base to the apex of the shoot and along the entire root, respectively. The number of true leaves per plantlet was counted, and the days to the appearance of the first true leaf were recorded from inoculation to leaf emergence. Vigor index was calculated according to Vashisth and Nagarajan (2010) as the product of the final germination percentage and the total seedling length (root + shoot), providing an integrated measure of both germination performance and early seedling growth.

2.7.3 Pigment accumulation

Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids (mg g^{-1}) were determined following the method of Hiscox and Israelstam (1979) with minor modifications. Leaves were collected and cut into thin strips. Leaf samples were incubated in a water bath containing 80% ethanol at 70 °C until the tissues were bleached. The absorbance of the solution was measured using a spectrophotometer at 645 and 663 nm for chlorophylls and at 470 nm for carotenoids. Pigment concentrations were calculated using the equations of Arnon (1949) for chlorophylls and the formula of Lichtenthaler (1987) for carotenoids, as follows:

Chlorophyll a (mg g^{-1}):

$$Chl_a = 12.7\ (A_{663}) - 2.69\ (A_{645}) \quad (2)$$

Chlorophyll b (mg g^{-1}):

$$Chl_b = 22.9\ (A_{645}) - 4.68\ (A_{663}) \quad (3)$$

Total Chlorophyll (mg g^{-1}):

$$Chl_{total} = 20.2\ (A_{645}) + 8.02\ (A_{663}) \quad (4)$$

Carotenoids (mg g^{-1}):

$$Carotenoids = \frac{1000\ (A_{470} - 3.27\ (Chl_a) - 104\ (Chl_b)}{227} \quad (5)$$

Where:

Chl_a = chlorophyll *a* concentration (mg g^{-1})
Chl_b = chlorophyll *b* concentration (mg g^{-1})
Chl_{total} = total chlorophyll concentration (mg g^{-1})
A_{645}, A_{663}, A_{470} = absorbance at the indicated wavelength

2.8. Statistical Analysis

Data were recorded, consolidated, tabulated, and statistically analyzed through analysis of variance (ANOVA) in a Randomized Complete Block Design (RCBD) with inoculation days as blocks. The Least Significant Difference (LSD) test was used to determine significant differences among treatment means. Statistical analysis was conducted using the computer software Statistical Tool for Agricultural Research (STAR) version 2.0.1, developed by the International Rice Research Institute (IRRI).

3. Results and Discussion

3.1 Germination Response

The germination response of coffee zygotic embryos to silver nanoparticles is presented in Table 1. In this study, the emergence of the cotyledon served as a key indicator of germination, although it is widely defined by radicle protrusion (Khan, et al., 2021).

Final germination percentage showed no statistical significance. In contrast, the coefficient of velocity of germination significantly increased at 0.5 mg L^{-1} AgNPs with values of 15.21, and is statistically comparable to 1.0 and 1.5 mg L^{-1} AgNPs with a CVG of 14.24 and 14.08, respectively. The results suggest improved vigor and more synchronized germination compared to MS alone, with 12.27 CVG. Mean germination time (MGT) also showed a significant reduction, with the shortest duration observed at 0.5 mg L^{-1} (6.58 days), considerably lower than MS alone (8.09 days) but statistically comparable to 1.0 and 1.5 mg L^{-1} AgNPs with an MGT of 7.05 and 7.11 days, respectively. This indicates a potential acceleration in metabolic activation and cell division due to AgNPs. Faraji and Sepehri (2019) stated that low concentrations of AgNPs can enhance germination by modulating enzymatic activity and stimulating reactive oxygen species (ROS) signaling.

These findings imply that AgNPs at 0.5 to 1.5 mg L^{-1} can improve the speed and uniformity of germination, with 0.5 mg L^{-1} being the most consistent. The results may be attributed to the influence of AgNPs on starch metabolism. Mahakham et al. (2017) found that nanopriming rice seeds with biosynthesized AgNPs significantly increased α-amylase activity, an enzyme responsible for converting starch into sugars during early germination. Their study reported a 2.5-to-2.6-fold increase in α-amylase activity and a 2.0-to-2.2-fold accumulation in total soluble sugars. Interestingly, chemically primed seeds using $AgNO_3$ or hydropriming did not produce similar results, which suggests a unique action of AgNPs. This supports the hypothesis that AgNPs improve early metabolic processes by promoting enzymatic activities and sugar mobilization.

Table 1. Germination response of arabica coffee to silver nanoparticle applications.

TREATMENTS	FGP (%)	CVG (-)	MGT (day)
MS Alone	70.00	12.37 ^{c}	8.09 ^{a}
MS + 0.1 mg L^{-1} AgNP	56.67	13.81 ^{b}	7.25 ^{b}
MS + 0.5 mg L^{-1} AgNP	74.17	15.21 ^{a}	6.58 ^{c}
MS + 1.0 mg L^{-1} AgNP	70.00	14.24 ^{ab}	7.05 ^{bc}
MS + 1.5 mg L^{-1} AgNP	80.83	14.08 ^{ab}	7.11 ^{bc}
P-value	0.0693	0.0072	0.0048
c.v (%)	11.91	4.55	4.41

Means with the same letter in a column are not significantly different (Fisher's LSD, $p < 0.05$); FGP (Final germination percentage), CVG (Coefficient of germination rate), and MGT (Mean germination time)

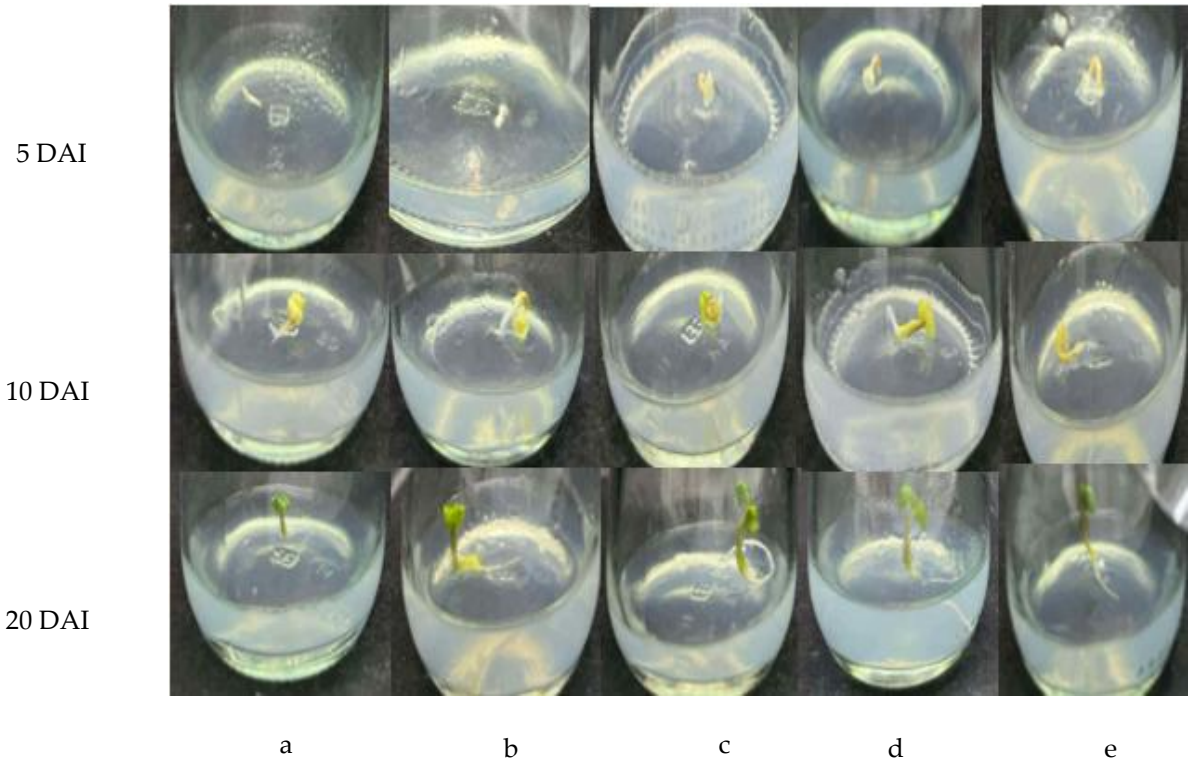

Figure 1. *In vitro* germination of arabica coffee after 5, 10, and 20 days of incubation (DAI) in AgNP-added medium: (a) MS Alone; (b) 0.1 mg L^{-1}; (c) 0.5 mg L^{-1}; (d) 1.0 mg L^{-1}; (e) 1.5 mg L^{-1}.

In this study, although the final germination percentage was not statistically significant, the marked reduction in MGT and significant CVG suggest a favorable physiological response in *C. arabica* embryos. These results are in agreement with previous findings that nanoparticles may serve as nanocatalysts, promoting early metabolic activity, enhancing antioxidant defense, and improving overall germination performance (Tymoszuk, 2021; Mahajan et al., 2022).

3.2 Shoot Length

The shoot length of *C. arabica* zygotic embryos as affected by varying concentrations of silver nanoparticles (AgNPs) is presented in Figure 2. At 4 weeks after incubation (WAI), no significant differences were observed among treatments, although early trends indicated a slight increase in shoot length in embryos treated with 1.0 mg L^{-1} AgNPs compared to the control, which continued at 6 WAI.

By 8 WAI, differences across treatments became statistically significant. The 1.0 mg L^{-1} treatment recorded the longest shoot length at 15.39 mm, whereas both MS alone, 0.1 mg L^{-1} and 0.5 mg L^{-1} treatments remained shorter (11.74 mm, 12.19 mm, and 12.50 mm, respectively). This trend was sustained through 10 and 12 WAI, where the 1.0 mg L^{-1} AgNPs continued to promote shoot elongation, reaching a final length of 17.21 mm at 12 WAI. The 1.5 mg L^{-1} treatment followed closely (16.05 mm), while MS alone consistently remained the shortest (13.72 mm). These results suggest that shoot growth in *C. arabica* zygotic embryos responded positively to AgNP supplementation, particularly at 1.0 mg L^{-1}. The consistent improvement in shoot length over time may reflect enhanced stimulation of cell division and elongation, or improved ion transport across growing tissues. Interestingly, while the 1.5 mg L^{-1} treatment also promoted shoot elongation, it did not outperform the 1.0 mg L^{-1} level, implying that higher concentrations may yield diminishing returns or even induce mild phytotoxic effects over prolonged exposure.

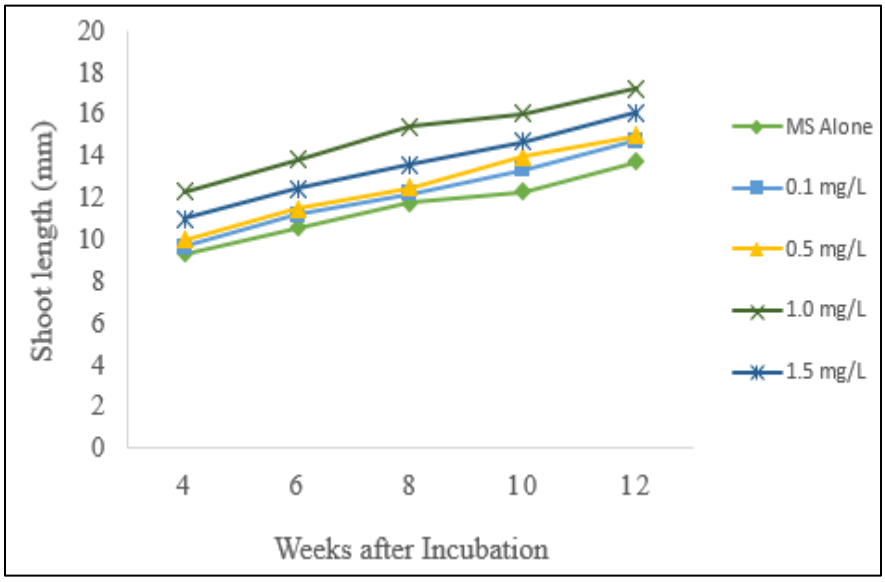

Figure 2. Shoot length (mm) of arabica coffee after 4, 6, 8, 10, and 12 weeks of incubation in AgNP-added medium

Earlier studies support this dose-dependent pattern of growth stimulation. For instance, Aguirre-Noyola (2025) stated that low doses of NPs can elicit positive responses in plants, while high doses can cause nanotoxicity. Sadak (2019) observed increased shoot length in fenugreek after AgNP application. In tomato (*Solanum lycopersicum*), Ansari et al. (2023) reported that 10 ppm AgNPs significantly improved its morphological characteristics, such as its shoot length. However, concentrations beyond 25 ppm resulted in reduced growth, suggesting a threshold beyond which the positive effects of AgNPs decline. These findings support the idea that AgNPs exhibit a hormetic effect, beneficial at low to moderate levels, but potentially inhibitory at higher concentrations. Although these concentrations are considerably higher than those used in the present study, the physiological response appears consistent. The observed enhancement in shoot elongation was attributed to increased pigments, soluble sugars, and proteins, which collectively support metabolic activity and cell proliferation.

3.3 Root Length

The effect of AgNPs on the root length of *C. arabica* zygotic embryos at different concentrations is presented in Figure 3. At 4 weeks after inoculation, significant differences were observed among the treatments. Applying 0.5 mg L^{-1} AgNPs produced the longest roots with a mean length of 26.20 mm, comparable to those embryos applied with 1.0 mg L^{-1} and 0.1 mg L^{-1} with root lengths of 23.53 mm and 22.70 mm, respectively. The control (MS alone) and 1.5 mg L^{-1} consistently recorded the shortest root length at 16.37 mm and 17.77 mm, respectively. Root elongation was still significantly influenced at 6 WAI, with the 0.5 mg L^{-1} and 1.0 mg L^{-1} treatments yielding 32.17 mm and 30.68 mm, respectively, compared to 19.83 mm in the control.

A similar trend continued at 10 WAI, where the same treatments recorded the highest values, 39.05 mm and 37.77 mm, respectively, while MS alone remained the lowest at 24.18 mm. Although statistical significance was no longer observed at 12 WAI, the 0.5 mg L^{-1} AgNPs produced a root length of 43.83 mm. The results indicate that silver nanoparticles enhanced root elongation over time, particularly at 0.5 mg L^{-1}. Accordingly, AgNPs enhanced root elongation through their effects on hormone signaling pathways.

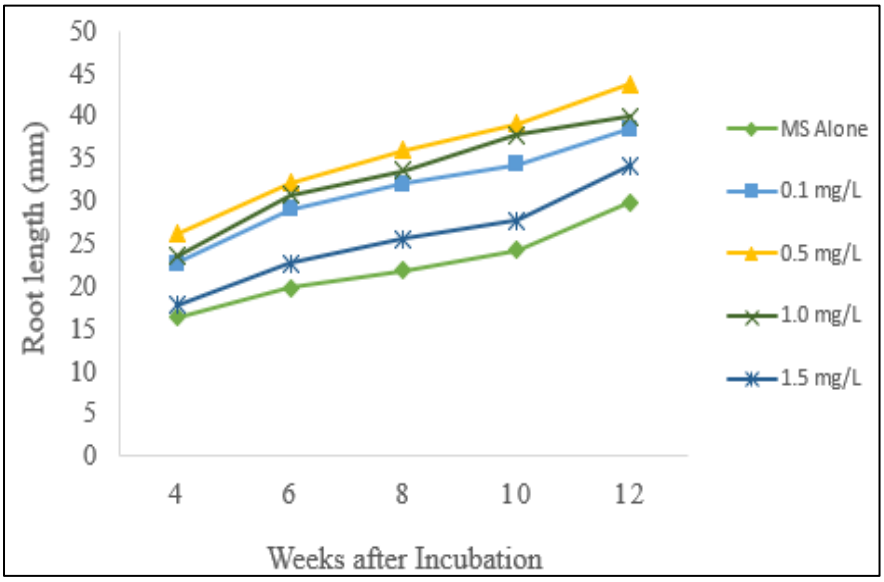

Figure 3. Root length (mm) of arabica coffee after 4, 6, 8, 10, and 12 weeks of incubation in AgNP-added medium

Syu et al. (2014) demonstrated that AgNPs can antagonize ethylene-mediated inhibition of root growth in Arabidopsis by reducing the expression of ACC synthase (ACS7) and ACC oxidase (ACO2). This interference with ethylene perception supports better elongation in developing tissues.

At lower concentrations, AgNPs have been found to stimulate moderate production of reactive oxygen species (ROS), particularly hydrogen peroxide (H_2O_2), which plays a role in activating the root meristem and promoting cell division. Wang et al. (2020) reported that low levels of ROS can function as signaling molecules involved in root development. In contrast, high concentrations lead to oxidative stress that disrupts the cell cycle, eventually hampering growth. This pattern of response was also evident in the present study, where a higher AgNP concentration (1.5 mg L^{-1}) led to reduced root length, suggesting a threshold level beyond which toxicity might occur.

The hormetic effect observed in this study, where lower concentrations of AgNPs enhanced root growth while higher concentrations suppressed it, is consistent with the results reported by Guzmán-Báez et al. (2021). They documented a similar response in tomato seedlings, where low levels of AgNPs promoted root length and number, attributed to enhanced auxin activity and nutrient uptake. Similar results were found in this study, where AgNPs, when applied at optimal concentrations, can enhance early root development in *C. arabica* zygotic embryos under *in vitro* conditions. However, exceeding this concentration may lead to a reverse effect due to oxidative stress or possible hormonal imbalances.

3.4 Morphological Traits

The morphological traits such as number of leaves, days to formation of true leaves and vigor index of *C. arabica* zygotic embryos cultured *in vitro* is presented in Table 2. The number of true leaves is a vital indicator of early seedling development in *C. arabica*. However, the application of varying levels of Ag nanoparticles showed no significant effect on the number of true leaves formed by the zygotic embryos. On average, the number of true leaves produced across treatments ranged from 2.84 to 3.27 leaves per seedling. Embryos treated with 1.0 mg L^{-1} obtained an average number of true leaves of 3.27, while the control obtained an average of 2.94 true leaves.

On the other hand, the application of silver nanoparticles significantly affected the mean number of days to the formation of the first true leaf. The earliest formation was observed in embryos treated with 0.5 mg L^{-1} AgNPs, which developed true leaves in just 54 days.

Table 2. Number of leaves, days to formation of true leaves, and vigor index of arabica coffee to silver nanoparticle applications.

Treatments	Number of true leaves	Days to formation of true leaves	Vigor index
MS Alone	2.94	62.00 ^{c}	305.33 ^{b}
MS + 0.1 mg L^{-1} AgNP	2.84	58.00 ^{b}	308.80 ^{b}
MS + 0.5 mg L^{-1} AgNP	3.27	54.00 ^{a}	437.39 ^{a}
MS + 1.0 mg L^{-1} AgNP	3.23	57.00 ^{b}	396.92 ^{ab}
MS + 1.5 mg L^{-1} AgNP	3.14	58.00 ^{b}	408.60 ^{a}
P-value	0.5697	0.0013	0.0394
c.v (%)	11.90	2.25	13.73

Means with the same letter in a column are not significantly different (Fisher's LSD, $p < 0.05$)

This was followed by embryos treated with 1.0 mg L^{-1}, 1.5 mg L^{-1}, and 0.1 mg L^{-1}, which formed true leaves at 57 and 58 days, respectively. MS without any supplementation of AgNPs delayed the onset of true leaf formation at 62 days. These findings show that AgNPs, particularly at 0.5 mg L^{-1}, promoted faster development of leaf structures in Arabica coffee embryos cultured *in vitro*. The earliest formation of true leaves observed in embryos treated with 0.5 mg L^{-1} AgNPs may be attributed to mechanisms similar to those reported by Manickavasagam et al. (2019) in rice calli, where low concentrations of AgNPs enhanced tissue development by regulating reactive oxygen species (ROS), improving antioxidant status, and modulating hormone levels such as abscisic acid and ethylene.

Moreover, the vigor index of *C. arabica* zygotic embryos were significantly influenced by the application of silver nanoparticles (Table 2). Embryos treated with 0.5 mg L^{-1} and 1.5 mg L^{-1} AgNPs obtained the highest vigor indices at 437.39 and 408.60, respectively. These values were significantly higher compared to the control (305.33) and 0.1 mg L^{-1} (308.80), while 1.0 mg L^{-1} (396.92) was statistically comparable to both groups.

The increased vigor index at 0.5 and 1.5 mg L^{-1} suggests enhanced seedling performance as a result of AgNP application. This improvement likely reflects faster germination and early seedling growth, potentially due to the stimulatory effects of silver nanoparticles on metabolic processes during in vitro development. Similar effects have been reported in rice seeds, where low concentrations of green-synthesized AgNPs enhanced seedling vigor by boosting α-amylase activity, increasing soluble sugar accumulation, upregulating aquaporin transporters, and optimizing reactive oxygen species (ROS) signaling, all of which support vigorous early seedling growth (Mahakham et al., 2017).

3.5 Photosynthetic Pigments

The application of silver nanoparticles (AgNPs) significantly influenced the photosynthetic pigment profile of *C. arabica* regenerants (Figure 4). The chlorophyll *a* content was highest at 0.5 mg L^{-1} AgNPs (5.90 mg g^{-1}), followed by 0.1 mg L^{-1} (5.67 mg g^{-1}), both significantly higher than the control (4.37 mg g^{-1}). For chlorophyll *b*, the 0.1 mg L^{-1} treatment recorded the highest value (2.48 mg g^{-1}), closely followed by 0.5 mg L^{-1} (2.40 mg g^{-1}), while the control was lower (2.03 mg g^{-1}). A similar pattern was observed for total chlorophyll, where 0.5 mg L^{-1} showed the highest content (8.29 mg g^{-1}), followed by 0.1 mg L^{-1} (8.15 mg g^{-1}). In contrast, the 1.5 mg L^{-1} treatment recorded the lowest total chlorophyll (3.04 mg g^{-1}), suggesting possible phytotoxicity at higher concentrations. Carotenoid content followed the same trend, where the highest value was observed at 0.5 mg L^{-1} (1.01 mg g^{-1}), followed by 0.1 mg L^{-1} (0.95 mg g^{-1}), whereas 1.5 mg L^{-1} showed the lowest level (0.46 mg g^{-1}), even lower than the control (0.67 mg g^{-1}). These results indicate that low to moderate concentrations of AgNPs enhance pigment biosynthesis, while excessive doses reduce it.

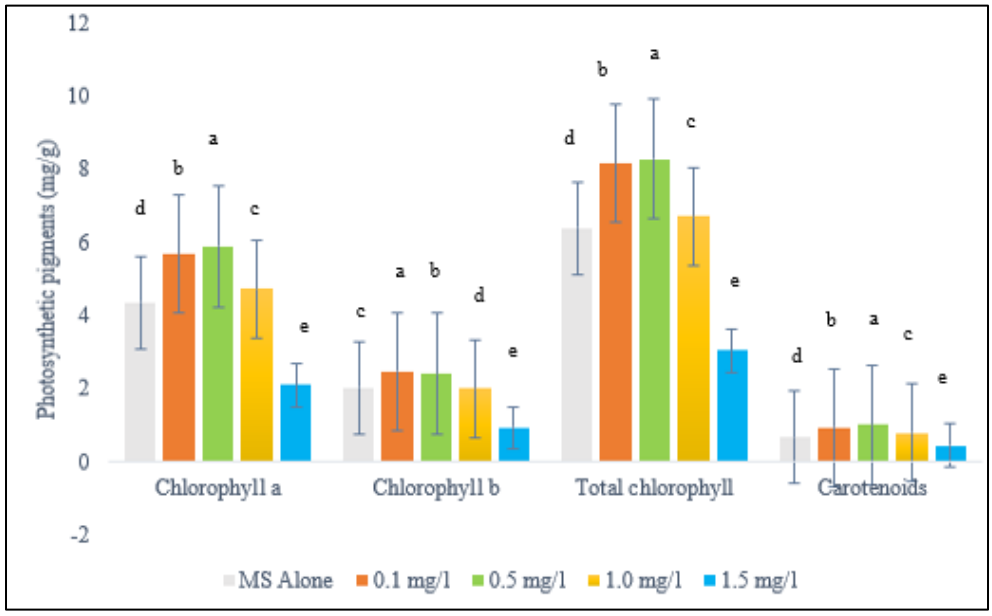

Figure 4. Photosynthetic pigments (mg g^{-1}) of arabica coffee in AgNP-added medium

Comparable findings were reported by Tejada-Alvarado et al. (2023), who showed that AgNPs significantly enhanced chlorophyll *a*, *b*, and carotenoids in *Ananas comosus* seedlings grown *in vitro* under $AlCl_3$-induced stress. In their study, pigment enhancement was most evident at moderate doses (e.g., 0.025 g L^{-1}), whereas higher concentrations reduced pigment accumulation. The authors attributed the stimulatory effect of AgNPs to their antioxidant activity, which neutralized reactive oxygen species (ROS) and preserved chloroplast integrity. Similarly, Sadak (2019) observed a dose-dependent response in *Trigonella foenum-graecum*, where foliar application of AgNPs up to 40 mg L^{-1} significantly increased chlorophyll and carotenoid contents. However, pigment levels declined at 60 mg L^{-1}. The stimulatory effect was attributed to enhanced indole acetic acid (IAA) activity and modulation of ethylene signaling, which are mechanisms that may also contribute to the responses observed in *C. arabica*, though further study is required.

4. Conclusion

The application of silver nanoparticles significantly enhanced germination, shoot and root elongation, vigor index, and pigment accumulation in arabica coffee embryos cultured *in vitro.* The optimal response was observed at 0.5–1.0 mg L^{-1}, where seedlings exhibited faster germination, more vigorous growth, and earlier true leaf development. These results suggest that AgNPs can serve as effective growth stimulants in coffee tissue culture by modulating physiological and metabolic processes. Further studies are recommended to validate these findings and to investigate potential synergistic effects with other nanoparticles or plant growth regulators for improved propagation of coffee.

5. Acknowledgement

The authors would like to sincerely thank the Department of Science and Technology – Accelerated Science and Technology Human Resource Development Program Scholarship (DOST-ASTHRDP) and the J.H. Cerilles State College for funding this research. Appreciation is also extended to the VSU Administration for providing laboratory facilities.

6. Competing Interests

The authors have declared that no competing interests exist.

7. Authors' Contributions

ALL Paler designed the study, conducted the experiments, performed the statistical analysis, and wrote the first draft of the manuscript. CC Arradaza supervised the study, provided guidance on the methodology, and critically reviewed the manuscript. All authors read and approved the final manuscript.

8. Use of Artificial Intelligence (AI)-Assisted Technology

The authors of this manuscript affirm that in the writing process of this work, no generative artificial intelligence (AI) or AI assisted technologies were used to generate scientific content, ideas, or references. AI tools were solely employed for enhancing readability and refining language, specifically Grammarly for grammar checking, QuillBot for paraphrasing guidance, and ChatGPT for phrasing suggestions. This use was strictly supervised and controlled by humans. Following the implementation of these AI-assisted technologies, the manuscript meticulously examined and revised by the authors to guarantee its precision, coherence, and scientific integrity. The authors are aware that AI can generate content that may sound authoritative yet might be incorrect, incomplete, or biased. In light of this, human judgment was used to review the manuscript thoroughly.

9. References

Aguirre-Noyola, J. L., Ramírez-Mosqueda, M. A., Cadena-Zamudio, J. D., Caamal-Velázquez, J. H., Cruz-Gutiérrez, E. J., & Armenta-Medina, A. (2025). Green-synthesized silver nanoparticles (AgNPs) enhance *in vitro* multiplication and rooting of strawberries (Fragaria × ananassa Duchesne). *BioTech*, *14*(2), 45. https://doi.org/10.3390/biotech14020045

Ansari, M., Ahmed, S., Abbasi, A., Khan, M. T., Subhan, M., Bukhari, N. A., & Abdelsalam, N. R. (2023). Plant-mediated fabrication of silver nanoparticles, process optimization, and impact on tomato plant. *Scientific Reports*, *13*(1), 18048. https://doi.org/10.1038/s41598-023-44706-2

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology, 24*(1), 1–15. https://doi.org/10.1104/pp.24.1.1

Bojórquez-Quintal, J. E. D. A., Sánchez-Cach, L. A., Gamboa-Tec, N. F., Quintal-Tun, F., Minero-García, Y., Martínez-Estévez, M., & Santos-Briones, D. C. L. (2011). Effect of plant growth regulators on *in vitro* germination of coffee zygotic embryos. *African Journal of Biotechnology, 10*(82), 19056–19065. https://doi.org/10.5897/AJB11.2667

Department of Agriculture. (2021). Philippine Coffee Industry Roadmap 2021–2025. https://pcaf.da.gov.ph/index.php/cir-coffee/

Faraji, J., & Sepehri, A. (2019). Ameliorative effects of TiO2 nanoparticles and sodium nitroprusside on seed germination and seedling growth of wheat under PEG-stimulated drought stress. *Journal of Seed Science, 41*(3), 309–317. https://doi.org/10.1590/2317-1545v41n3207807

Guzmán-Báez, G. A., Trejo-Téllez, L. I., Ramírez-Olvera, S. M., Salinas-Ruíz, J., Bello-Bello, J. J., Alcántar-González, G., & Gómez-Merino, F. C. (2021). Silver nanoparticles increase nitrogen, phosphorus, and potassium concentrations in leaves and stimulate root length and number of roots in tomato seedlings in a hormetic manner. *Dose-Response, 19*(4), 15593258211044576. https://doi.o/10.1177/15593258211044576

Gómez, J. H., Benavides, P., Maldonado, J. D., Jaramillo, J., Acevedo, F. E., & Gil, Z. N. (2023). Flower-visiting insects ensure coffee yield and quality. *Agriculture, 13*(7), 1392. https://doi.org/10.3390/agriculture13071392

Hiscox, J. D., & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany, 57*(12), 1332–1334. https://doi.org/10.1139/b79-163

International Coffee Organization. (2022). Annual review of coffee production 2021–2022. https://www.ico.org/documents/cy2022-23/annual-review-2021-2022-e.pdf

Khan, I., Awan, S. A., Raza, M. A., Rizwan, M., Tariq, R., Ali, S., & Huang, L. (2021). Silver nanoparticles improved plant growth and reduced the sodium and chlorine accumulation in pearl millet: A life cycle study. *Environmental Science and Pollution Research, 28*(11), 13712–13724. https://doi.org/10.1007/s11356-020-11624-1

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. In L. Packer & R. Douce (Eds.), *Methods in enzymology* (Vol. 148, pp. 350–382). Academic Press. https://doi.org/10.1016/0076-6879(87)48036-1

Mahajan, S., Kadam, J., Dhawal, P., Barve, S., & Kakodkar, S. (2022). Application of silver nanoparticles in in-vitro plant growth and metabolite production: Revisiting its scope and feasibility. *Plant Cell, Tissue and Organ Culture, 150*(1), 15–39. https://doi.org/10.1007/s11240-022-02297-7

Mahakham, W., Sarmah, A. K., Maensiri, S., & Theerakulpisut, P. (2017). Nanopriming technology for enhancing germination and starch metabolism of aged rice seeds using phytosynthesized silver nanoparticles. *Scientific Reports, 7*(1), 8263. https://doi.org/10.1038/s41598-017-08669-5

Manickavasagam, M., Pavan, G., & Vasudevan, V. (2019). A comprehensive study of the hormetic influence of biosynthesized AgNPs on regenerating rice calli of indica cv. IR64. *Scientific Reports, 9*(1), 8821. https://doi.org/10.1038/s41598-019-45263-4

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3).

Philippine Statistics Authority. (2018.). Other crops: Volume of production, by region and by province, 1990–2018; Other crops: Area planted/harvested, by region and by province, 1990–2018. Retrieved March 3, 2024, from http://openstat.psa.gov.ph/

Pinto, R. T., Ribeiro, L. O., Torres, L. F., Freitas, N. C., Barbosa, S., Santos, B. R., & Paiva, L. V. (2018). Coffee synthetic seed production is influenced by somatic embryo development stage and nutritional factors related to the encapsulation matrix. *In XXX International Horticultural Congress IHC2018: II International Symposium on Micropropagation and In Vitro Techniques* (Vol. 1285, pp. 31–38). International Society for Horticultural Science. https://doi.org/10.17660/ActaHortic.2020.1285.5

Prasad, A., Sidhic, J., Sarbadhikary, P., Narayanankutty, A., George, S., George, B. P., & Abrahamse, H. (2024). Role of metal nanoparticles in organogenesis, secondary metabolite production and genetic transformation of plants under in vitro condition: a comprehensive review. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *158*(2), 33.

Sadak, M. S. (2019). Impact of silver nanoparticles on plant growth, some biochemical aspects, and yield of fenugreek plant (*Trigonella foenum-graecum*). *Bulletin of the National Research Centre, 43*(1), 1–6. https://doi.org/10.1186/s42269-019-0142-1

Sami, F., Siddiqui, H., & Hayat, S. (2020). Impact of silver nanoparticles on plant physiology: A critical review. In S. Hayat & J. Pichtel (Eds.), Sustainable agriculture reviews 41: Nanotechnology for plant growth and development (pp. 111–127). *Springer*. https://doi.org/10.1007/978-3-030-34541-1_4

Şener, S., & Sayğı, H. (2023). The role of silver nanoparticles in response of *in vitro* boysenberry plants to drought stress. *Horticulturae, 9*(11), 1177. https://doi.org/10.3390/horticulturae9111177

Syu, Y. Y., Hung, J. H., Chen, J. C., & Chuang, H. W. (2014). Impacts of size and shape of silver nanoparticles on Arabidopsis plant growth and gene expression. *Plant Physiology and Biochemistry, 83*, 57–64. https://doi.org/10.1016/j.plaphy.2014.07.010

Tamimi, S. M., & Othman, H. (2023). Silver nanoparticles for enhancing the efficiency of micropropagation of banana (*Musa acuminata* L.). *Tropical Life Sciences Research, 34*(2), 161–173. https://doi.org/10.21315/tlsr2023.34.2.10

Tejada-Alvarado, J. J., Meléndez-Mori, J. B., Ayala-Tocto, R. Y., Goñas, M., & Oliva, M. (2023). Influence of silver nanoparticles on photosynthetic pigment content and mineral uptake in pineapple seedlings grown *in vitro* under aluminum stress. *Agronomy, 13*(5), 1186. https://doi.org/10.3390/agronomy13051186

Tung, H. T., Thuong, T. T., Cuong, D. M., Luan, V. Q., Hien, V. T., Hieu, T., & Nhut, D. T. (2021). Silver nanoparticles improved explant disinfection, *in vitro* growth, runner formation and limited ethylene accumulation during micropropagation of strawberry (Fragaria × ananassa). *Plant Cell, Tissue and Organ Culture, 145*(2), 393–403. https://doi.org/10.1007/s11240-021-01986-6

Tymoszuk, A. (2021). Silver nanoparticles' effects on *in vitro* germination, growth, and biochemical activity of tomato, radish, and kale seedlings. *Materials, 14*(18), 5340. https://doi.org/10.3390/ma14185340

Vashisth, A., & Nagarajan, S. (2010). Effect on germination and early growth characteristics in sunflower (*Helianthus annuus*) seeds exposed to static magnetic field. *Journal of Plant Physiology, 167*(2), 149–156. https://doi.org/10.1016/j.jplph.2009.08.011

Wang, L., Sun, J., Lin, L., Fu, Y., Alenius, H., Lindsey, K., & Chen, C. (2020). Silver nanoparticles regulate Arabidopsis root growth by concentration-dependent modification of reactive oxygen species accumulation and cell division. *Ecotoxicology and Environmental Safety*, 190, 110072. https://doi.org/10.1016/j.ecoenv.2019.110072

Wintgens, J. N., & Zamarripa, A. (2004). Coffee propagation. In J. N. Wintgens (Ed.), Coffee: Growing, processing, sustainable production (pp. 87–136). Wiley-VCH.