RAMOS, M. C. P.; MOREIRA, R. A.; RODRIGUES, M. A.; PIO, L. A. S.; BUENO FILHO, J. S. S.; BARATTI, A. C. C. In vitro cultivation of banana in a culture medium supplemented with selenium. **Revista de Agricultura Neotropical**, Cassilândia-MS, v. 13, n. 1, e10012, 2026. ISSN 2358-6303. DOI: https://doi.org/10.32404/rean.v13i1.10012

In vitro cultivation of banana in a culture medium supplemented with selenium

Martha Cristina Pereira Ramos[1], Renata Amato Moreira[1], Mariane Aparecida Rodrigues[1], Leila Aparecida Salles Pio[1], Julio Sílvio de Sousa Bueno Filho[1], Ana Claudia Costa Baratti[1]

[1] Lavras Federal University, Lavras, Minas Gerais, Brazil. E-mail: marthinha.ramos@yahoo.com.br, renata_amato@hotmail.com, marianerodriguesst@gmail.com, leila.pio@ufla.br, jssbueno@ufla.br, anaclaudia.costa@ufla.br

Received: 10/16/2025; Accepted: 04/23/2026.

ABSTRACT

Selenium (Se) can be supplemented in the human diet through the consumption of foods rich in this element. However, these foods are often inaccessible to low-income populations. In this context, the enrichment of staple food crops can increase the nutritional quality of the product and contribute to efforts to reduce malnutrition. Thus, during in vitro culture, the culture medium can be enriched with elements such as Se to biofortify the plantlets. The objective of this study was to evaluate the production of Se-biofortified micropropagated banana plantlets. 'Prata Anã' Gorutuba banana plants were cultured in vitro and subsequently acclimatized. The experiment was conducted in a completely randomized design, and the treatments consisted of the addition of sodium selenate at Se concentrations of 0, 10, 20, 30, 40, and 50 µmol L^{-1}, with five replicates and six culture tubes per replicate. Growth parameters, photosynthetic pigment contents, biochemical parameters, and Se accumulation were evaluated in each treatment at 30 days of in vitro cultivation. The number of leaves, shoot length, and stem base diameter were evaluated at 90 days of greenhouse acclimatization. The intermediate concentration of 20 µmol L^{-1} Se was the most suitable for obtaining plants with greater Se accumulation without adversely affecting phytotechnical traits and resulted in greater photosynthetic activity. Higher Se concentrations increased Se accumulation but negatively affected plant growth, indicating a dose-dependent toxic effect at elevated concentrations. During acclimatization, intermediate Se concentrations maintained a better balance between biofortification efficiency and plantlet quality. Photosynthetic pigments responded positively to lower Se concentrations, whereas antioxidant enzyme activity was not significantly affected. Overall, Se biofortification through micropropagation is feasible when appropriate concentrations are used to maintain plant quality and physiological stability.

Keywords: Antioxidant enzymes, Gorutuba clone, *Musa* spp., Sodium selenate, Tissue culture.

Cultivo in vitro de bananeira em meio de cultura suplementado com selênio

RESUMO

O selênio (Se) pode ser suplementado na dieta humana por meio do consumo de alimentos ricos nesse elemento. No entanto, esses alimentos são frequentemente inacessíveis às populações de baixa renda. Nesse contexto, o enriquecimento de culturas alimentares básicas pode aumentar a qualidade nutricional do produto e contribuir para os esforços de redução da desnutrição. Assim, durante o cultivo in vitro, o meio de cultura pode ser enriquecido com elementos como o Se para biofortificar as plântulas. O objetivo deste estudo foi avaliar a produção de plântulas de bananeira micropropagadas e biofortificadas com Se. Plantas de bananeira 'Prata Anã' Gorutuba foram cultivadas in vitro e, posteriormente, aclimatizadas. O experimento foi conduzido em delineamento inteiramente casualizado, e os tratamentos consistiram na adição de selenato de sódio em concentrações de Se de 0, 10, 20, 30, 40 e 50 µmol L^{-1}, com cinco repetições e seis tubos de cultivo por repetição. Parâmetros de crescimento, teores de pigmentos fotossintéticos, parâmetros bioquímicos e acúmulo de Se foram avaliados em cada tratamento aos 30 dias de cultivo in vitro. O número de folhas, o comprimento da parte aérea e o diâmetro da base do caule foram avaliados aos 90 dias de aclimatização em casa de vegetação. A concentração intermediária de 20 µmol L^{-1} de Se

foi a mais adequada para a obtenção de plantas com maior acúmulo de Se, sem afetar adversamente os caracteres fitotécnicos, e resultou em maior atividade fotossintética. Concentrações mais elevadas de Se aumentaram o acúmulo desse elemento, mas afetaram negativamente o crescimento das plantas, indicando efeito tóxico dependente da dose em concentrações elevadas. Durante a aclimatização, concentrações intermediárias de Se mantiveram melhor equilíbrio entre a eficiência da biofortificação e a qualidade das plântulas. Os pigmentos fotossintéticos responderam positivamente às menores concentrações de Se, enquanto a atividade das enzimas antioxidantes não foi significativamente afetada. De modo geral, a biofortificação com Se por meio da micropropagação é viável quando concentrações adequadas são utilizadas para manter a qualidade das plantas e a estabilidade fisiológica.

Palavras-chave: Enzimas antioxidantes, Clone Gorutuba, *Musa* spp., Selenato de sódio, Cultura de tecidos.

1. Introduction

Banana (*Musa* spp.) has substantial socioeconomic importance worldwide and is one of the main fruit crops cultivated in tropical and subtropical regions. The fruit is widely consumed across all social classes and is highly important for food security, income generation, and the livelihoods of millions of producers, particularly in developing countries (Bermeo-Fúquene et al., 2025). Banana production increased from approximately 97 million metric tons in 2008 to nearly 120 million metric tons in 2023, with 5.2 million hectares under cultivation, according to the Food and Agriculture Organization of the United Nations (FAO) (FAO, 2024).

Banana is propagated vegetatively, and micropropagation is used in commercial banana production systems (Couto et al., 2022; Costa et al., 2021). However, little is known about Se enrichment during micropropagation or its effects on plant growth and development.

Selenium (Se) is an essential nutrient for humans and is involved in several biochemical reactions that increase antioxidant activity (Hernández-Hernández et al., 2019; Rayman, 2002). Furthermore, this element is a component of proteins and amino acids that are important for cell growth and function (Puccinelli et al., 2017). Se deficiency in humans can impair immune and cognitive function and may be associated with heart disease, Alzheimer's disease, hypothyroidism, fertility problems, and cancer (Lopes et al., 2017; Cardoso et al., 2015; Rayman, 2002). Recent studies focused on demonstrating the effects of Se on improving immunity and, consequently, minimizing the effects of Coronavirus Disease 2019 (COVID-19) in the human body (Gois et al., 2020), mainly through host antioxidant defense (Guillin et al., 2019). In this context, research aimed at increasing Se availability in foods for human consumption has substantial social, nutritional, and economic importance.

Se intake in the human diet can be increased through the consumption of foods with high Se concentrations, such as fish, meat, nuts, bread, and cereals (Fairweather-Tait et al., 2011). However, such foods are frequently unavailable to populations with limited economic resources, who are more susceptible to hidden hunger. The enrichment of staple food crops during growth with elements and vitamins that improve nutritional quality can contribute to reducing malnutrition; this process is known as biofortification.

In vitro Se enrichment can accelerate the development of nutritionally improved plants because environmental variables can be controlled more efficiently and exposure to stress factors can be reduced (Tangjaidee et al., 2023). In addition, young tissues may absorb and distribute minerals more efficiently.

Plant biofortification studies are generally evaluated through the final product consumed as food (Ofori et al., 2022). Recent studies indicate that Se biofortification in young plants or seedlings can be effective. According to Quispe et al. (2025), Se seedling priming in broccoli favored mineral accumulation without compromising vegetative growth, whereas Namorato et al. (2025) demonstrated that different Se sources (Se-enriched urea and Se-enriched ammonium sulfate) increased grain Se concentration in common bean, with variation depending on genotype.

This study tested the hypothesis that selenium supplementation during in vitro plantlet production results in significant Se accumulation in banana plants without impairing growth parameters. The objective of this study was to evaluate the production of Se-biofortified micropropagated banana plantlets.

2. Material and Methods

The experiment was conducted in 2020 at the Tissue Culture Laboratory of the Federal University of Lavras (Universidade Federal de Lavras – UFLA), Lavras, Minas Gerais, Brazil (21°14'S, 44°59'W, 919 m altitude; Cwa climate). Explants were obtained from 'Prata Anã' banana plantlets, clone Gorutuba, provided by the Brazilian Agricultural Research Corporation (Embrapa Cassava and Tropical Fruits) and were cultured in semisolid MS medium (Murashige and Skoog, 1962)

supplemented with 25 g L^{-1} sucrose and 6 g L^{-1} agar, with the pH adjusted to 5.7 ± 0.1 before autoclaving. Test tubes containing 15 mL of culture medium were used.

A preliminary test was conducted to define the treatments and concentrations to be used in the experiment, with treatments consisting of two Se sources, sodium selenate and sodium selenite, at Se concentrations of 0, 25, 50, 75, and 100 µmol L^{-1}, with four replicates and two tubes per replicate. Based on the preliminary test results, the treatment with selenite was excluded because the observed phytotoxicity caused plantlet death.

The experiment was conducted in a completely randomized design, and the treatments consisted of the addition of sodium selenate at Se concentrations of 0, 10, 20, 30, 40, and 50 µmol L^{-1}, with five replicates and six culture tubes per replicate. The tubes were maintained in a growth room at 25 ± 1 °C for 30 days. For plant acclimatization, the micropropagated plantlets were transferred to a greenhouse with 50% shade at ambient temperature, and irrigation was performed manually once daily. Ten plantlets from each treatment were placed in trays containing a commercial substrate (Plantmax®), and the plants remained in this environment for 90 days.

Growth parameters were evaluated at 30 days of in vitro cultivation, including leaf number, number of roots, shoot length, and main root length, measured with a ruler, and shoot fresh and dry mass, measured on an analytical balance. The number of leaves, shoot length, and stem base diameter were measured at 90 days of greenhouse acclimatization using a digital caliper. Se analyses were performed in the Geochemistry Laboratory of the Department of Soil Science at UFLA. Se contents in banana plantlets were determined at 30 days of in vitro cultivation and at 90 days of greenhouse acclimatization. Extracts were prepared from 100 mg of dry mass by acid digestion with concentrated HNO_3 in closed vessels in a microwave oven, according to Method 3051A of the United States Environmental Protection Agency (USEPA) (U.S. EPA, 2007).

Se content in the digested solutions was measured using graphite furnace atomic absorption spectrometry with Zeeman background correction and an electrodeless discharge lamp for Se (AAnalyst™ 800 AAS, PerkinElmer). The calibration curve for Se measurement was prepared from a standard solution containing 1 g kg^{-1} Se (≥98% purity; Fluka, Buchs, Switzerland). A standard reference material from the Institute for Reference Materials and Measurements (white clover, BCR-402; IRMM, Geel, Belgium) and a blank sample were included in each digestion batch for quality assurance and quality control in Se measurement. The mean recovery obtained for the standard reference material was 96.78 ± 1.88% (n = 13).

Enzymatic and photosynthetic pigment analyses were performed only at 30 days of in vitro cultivation (Ahmed and Anis, 2014; Rahman et al., 2024). Banana leaves from the plantlets were collected, immediately placed in liquid nitrogen, and stored at −80 °C until evaluation of enzymatic activities. For the analysis, 0.2 g of fresh leaf tissue was homogenized in 1.5 mL of extraction buffer (0.1 mol L^{-1} potassium phosphate, pH 7.8; 0.1 mmol L^{-1} EDTA, pH 7.0; 0.01 mol L^{-1} ascorbic acid; and 22 mg of polyvinylpolypyrrolidone [PVPP]) with a mortar and pestle precooled with liquid nitrogen, according to Biemelt et al. (1998). The suspension was then centrifuged at 13,000 × g for 10 min at 4 °C, and the supernatant was collected to analyze superoxide dismutase (SOD) (Giannopolitis and Ries, 1977), catalase (CAT) (Havir and McHale, 1989), and ascorbate peroxidase (APX) (Nakano and Asada, 1981) activities.

Three biological replicates, each analyzed in triplicate, were used for each treatment, and readings were obtained using enzyme-linked immunosorbent assay (ELISA) spectrometry. The photosynthetic pigments analyzed were chlorophyll a, chlorophyll b, and carotenoids. The analyses were performed at the Tissue Culture Laboratory of the Department of Agriculture at UFLA, following the methodology described by Lichtenthaler and Buschmann (2001).

For pigment extraction, 0.1 g of fresh leaves from banana plantlets was weighed, homogenized with 5 mL of 80% (v/v) acetone, and filtered through glass wool; the volume was then brought to 10 mL with 80% acetone. Immediately after this procedure, readings were obtained at 663.2, 646.8, and 470 nm. The entire procedure was performed in the dark to prevent chlorophyll degradation (Lichtenthaler and Buschmann, 2001). Chlorophyll and carotenoid contents were calculated using the following equations: chlorophyll a = [(12.25 × A663.2) − (2.79 × A646.8)], chlorophyll b = [(21.5 × A646.8) − (5.1 × A663.2)], total chlorophyll = (a + b), and carotenoids = [((1000 × A470) − (1.82 × A663.2) − (85.02 × A646.2))/198] (Lichtenthaler, 1987; Lichtenthaler and Buschmann, 2001); the results were expressed as mg pigment g^{-1} fresh leaf tissue. Three biological replicates, each analyzed in triplicate, were used for each treatment.

Data were subjected to analysis of variance ($p < 0.05$), and linear and quadratic regression models were fitted for selenate concentrations under laboratory and greenhouse conditions using RStudio software (RStudio Team, 2012).

3. Results and Discussion

For the growth variables of in vitro plantlets, shoot length, fresh mass, and Se content in fresh and dry mass differed significantly among selenate concentrations, with a linear fit. Root length showed a quadratic fit. No significant differences were detected for leaf number (p = 0.7863) or root number (p = 0.2894).

For plants acclimatized in the greenhouse for 90 days, height (p = 0.2517) and leaf number (p = 0.4150) showed no significant differences. Stem base diameter showed a quadratic fit, whereas Se content in fresh and dry mass showed a linear fit, with differences among Se concentrations.

In the sequential analysis of traits with significant differences, the t test was performed to indicate increases or decreases in response to selenate concentrations and interactions. The intercept values in each linear model represented the overall mean of each variable plus the effect of the sum of the factors (Σikri = 0). For each variable that was significant by the F test, the estimates of factor effects or their associations in the t test represented an increase or reduction in the response variable when concentrations were increased from the lower concentration to the immediately higher concentration. Shoot length and root length showed negative effects. Se content in dry mass showed a positive effect in response to Se concentrations.

Increasing Se concentrations in the medium caused progressive reductions in plant length and root length and a significant decline in dry mass. However, low Se concentrations had beneficial effects on plant growth; Se is known to interfere with xyloglucan metabolism and affect cell expansion in plants and, consequently, plant growth (Zhou et al., 2018).

A similar effect was observed by Hawrylak-Nowak (2013) in lettuce plants under hydroponic conditions; a deleterious effect of the highest Se concentrations was observed on roots, shoots, and leaf area. Domokos-Szabolcsy et al. (2014) tested the tolerance of two giant sugarcane ecotypes to red elemental nanoselenium and selenate, and the Blossom ecotype showed toxic symptoms from 20 mg L^{-1} Se. Furthermore, Dall'Acqua et al. (2019) also found that higher concentrations, such as 40 µM, were associated with reduced leaf biomass.

In Figures 1, 2, 3, and 4, the 95% confidence intervals for the traits evaluated in each graph are represented by the hatched areas. The mean length of banana plantlets was greater at the lowest selenate concentrations (Figure 1). Control plants had higher values than plants grown in treatments containing selenate; however, plant growth remained substantial, even in the presence of Se, without severe damage.

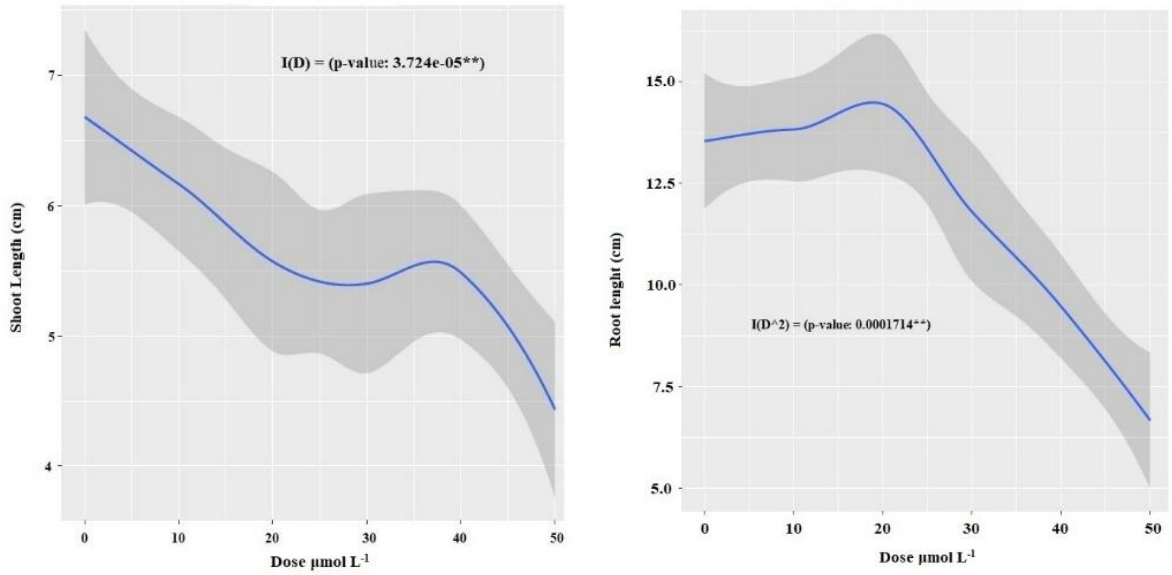

Figure 1. 95% confidence intervals for mean shoot length and root length of 'Prata Anã' Gorutuba banana plantlets cultivated in vitro at different sodium selenate concentrations.

The 10 and 20 µmol L^{-1} Se concentrations resulted in the greatest root lengths, with a marked decrease observed at the subsequent concentrations; thus, root development decreased as the applied concentration increased (Figure 1). The results indicate that Se, at lower concentrations, may be beneficial to root growth.

Fresh mass was higher at lower concentrations, with a decrease beginning at 30 µmol L^{-1}, whereas the highest concentration, 50 µmol L^{-1}, resulted in the lowest fresh mass value (Figure 2).

Among the growth traits evaluated in acclimatized plants, only stem base diameter was significantly affected. Furthermore, as observed for plants grown under laboratory conditions, the highest values were obtained at intermediate concentrations (Figure 3),

whereas the highest concentration reduced stem base diameter.

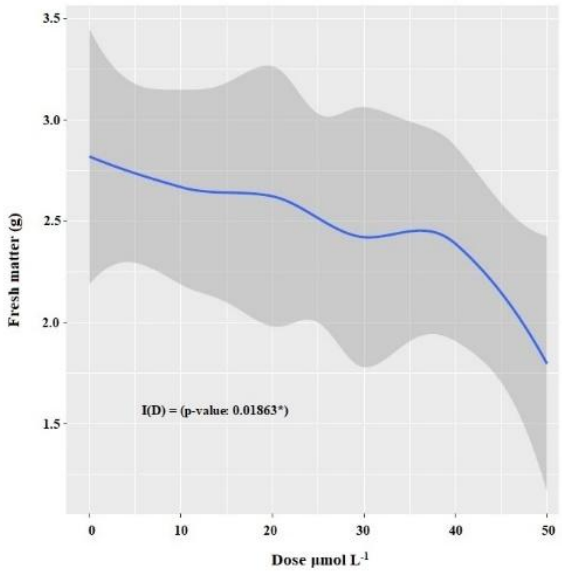

Figure 2. 95% confidence intervals for mean fresh mass of 'Prata Anã' Gorutuba banana plantlets cultivated in vitro at different sodium selenate concentrations.

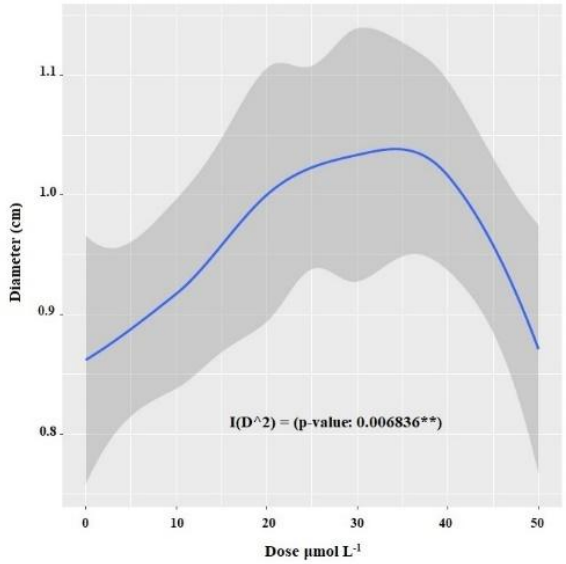

Figure 3. 95% confidence intervals for mean stem base diameter of 'Prata Anã' Gorutuba banana plantlets acclimatized in a greenhouse for 90 days after cultivation at different sodium selenate concentrations.

Se can benefit plantlet quality in terms of both phytotechnical performance and plant protection. Studies have shown that low Se concentrations protect plants from abiotic stress (Feng et al., 2013). The effects of Se on plant growth can be attributed mainly to its antioxidant function, mediated by selenoproteins; these compounds protect plants from oxidative damage (Souza et al., 2019).

Sharma and Uttam (2020) used spectral signature analysis and infrared spectroscopy to describe the involvement of Se in modulating cell wall and membrane structure in wheat seedlings and reported that high Se concentrations significantly reduced the biochemical components of wheat seedling cell walls and leaf membranes, such as cellulose, hemicellulose, pectin, lignin, amino acids, proteins, and lipids. Thus, this element should be recommended at lower amounts to produce high-quality plantlets.

Se content in fresh and dry mass as a function of Se concentrations (Figure 4) can be used to define a concentration that promotes plantlet development and results in greater accumulation of the element.

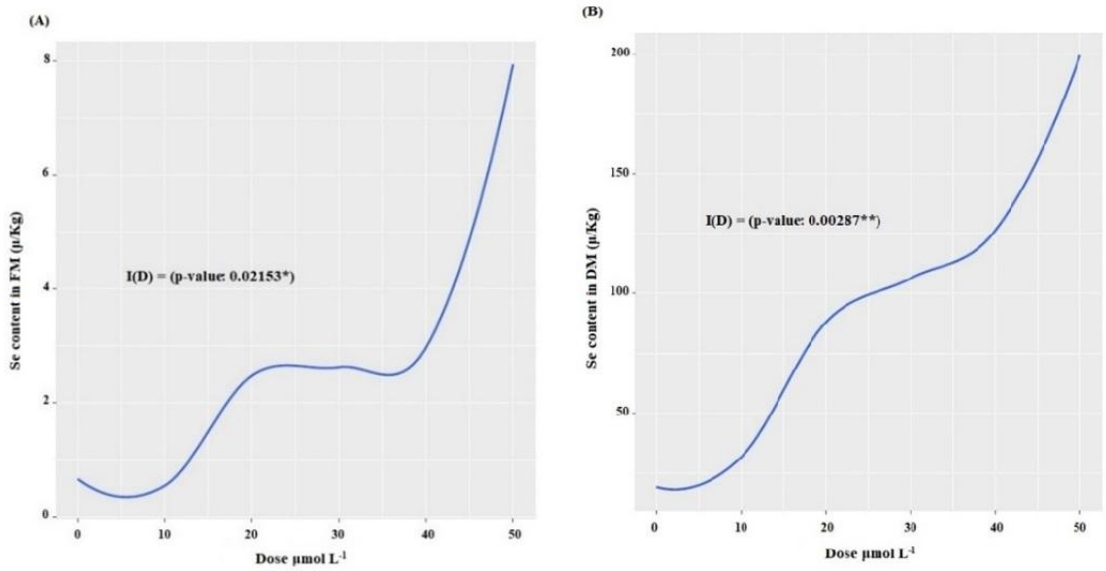

Figure 4. Se content in (A) fresh mass and (B) dry mass of 'Prata Anã' Gorutuba banana plantlets cultivated in vitro with the addition of sodium selenate.

Se accumulation in fresh and dry mass increased with increasing Se concentrations, with the concentration of 50 µmol L^{-1} resulting in the greatest Se accumulation. However, this information should be interpreted together with the results for phytotechnical traits because higher concentrations may impair plantlet development.

When Se accumulation and phytotechnical traits were considered together, intermediate concentrations, such as 20 µmol L^{-1}, showed the most favorable results from both perspectives. Thus, plants with greater Se accumulation due to the addition of excessive Se showed delayed growth and development compared with plants that received lower Se concentrations.

Se content in plants acclimatized in the greenhouse increased as the concentration increased (Figure 5), except for a decrease at 30 µmol L^{-1}. This result should be considered together with growth traits because, although plants accumulated more Se, reductions in phytotechnical performance were observed. Thus, 20 µmol L^{-1} appeared to be the most suitable concentration under greenhouse conditions, resulting in considerable stem base diameter and Se content.

Regarding Se quantification, plantlets under laboratory conditions showed higher Se values than greenhouse-acclimatized plants (Table 1).

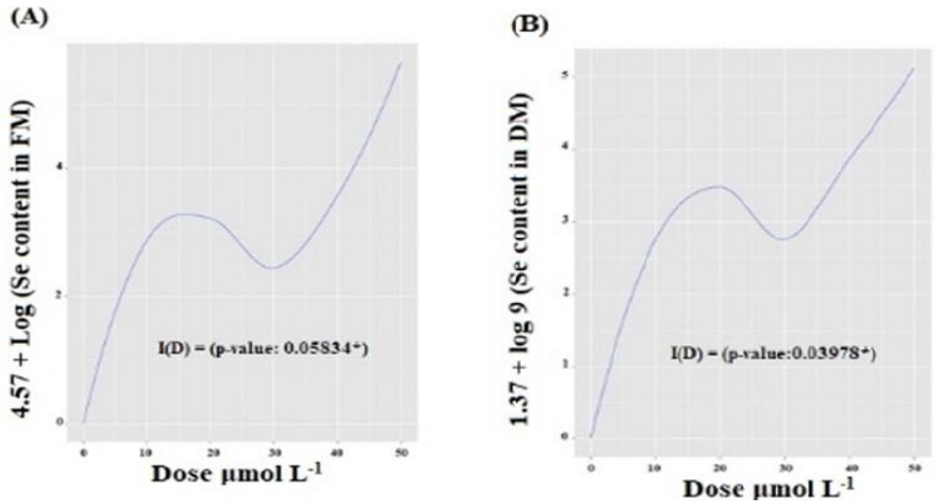

Figure 5 - Se content in (A) fresh mass and (B) dry mass of 'Prata Anã' Gorutuba banana plantlets acclimatized in a greenhouse for 90 days after sodium selenate addition.

Table 1. Selenium (Se) accumulation in the dry mass of 'Prata Anã' Gorutuba banana plants (µg kg^{-1} dry mass) at 30 days of in vitro cultivation and at 90 days of greenhouse acclimatization.

Se concentrations (µmol L^{-1})	Plants under in vitro cultivation	Plants under greenhouse acclimatization
0	19.15	0.25
10	31.63	3.99
20	88.01	8.26
30	106.35	3.99
40	126.34	12.98
50	199.50	42.86

High dilution of this element occurred during plant growth and development. Thus, for biofortification through micropropagation, obtaining fruits with adequate Se content may require Se supplementation after field planting. Moreover, when plants did not receive supplementation with Se after field planting, the element absorbed in vitro remained present in plant structures but at very low concentrations.

Untreated banana plants were characterized by low Se concentrations in their leaves under laboratory and greenhouse conditions, with values of 19.15 and 0.25 µg kg^{-1} dry mass, respectively (Table 1). However, when 10 µmol L^{-1} Se was used, leaf Se concentration increased 1.65-fold under in vitro cultivation and 15.96-fold during acclimatization. Higher Se concentrations (40 and 50 µmol L^{-1}) contributed to a significant increase in leaf Se concentration. When Se was added under laboratory conditions, its level in the leaves was 4.6-fold higher than in greenhouse-acclimatized plants at the same Se concentrations.

The antioxidant enzyme complex was evaluated only in in vitro plantlets, and no significant differences were detected for the three enzymes analyzed. For photosynthetic pigments, chlorophyll a, chlorophyll b, and total chlorophyll showed similar responses, and the treatment with 10 µmol L^{-1} resulted in the highest chlorophyll values. This response differed from that of carotenoids, for which the 30 µmol L^{-1} treatment showed higher values.

Quadratic models were fitted for chlorophylls, with decreasing responses and slight upward curvature. In contrast, carotenoids showed an approximately stable response model across Se concentrations (Figure 6).

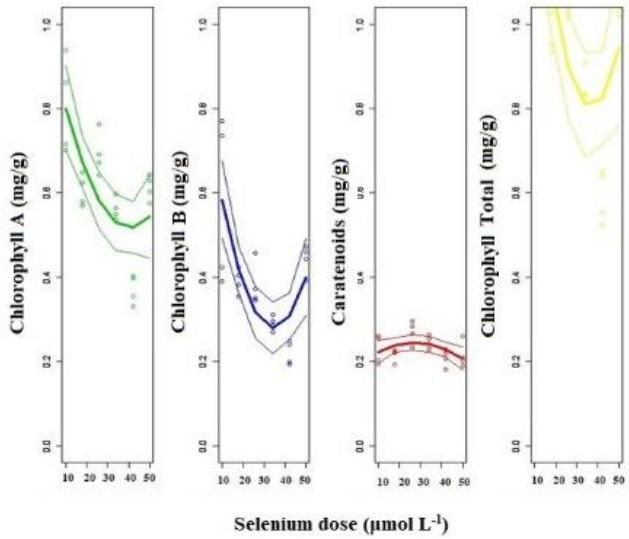

Figure 6. Photosynthetic pigments, including chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids, in 'Prata Anã' Gorutuba banana plants cultivated in vitro with sodium selenate addition.

Photosynthetic pigment concentrations were also higher at lower selenate concentrations; thus, Se in plants may contribute substantially to plant protection, mainly against biotic and abiotic stress, with benefits such as improved photosynthetic performance (Natasha et al., 2018). These modifications may indicate adaptive plasticity of the species under these conditions. Djanaguiraman et al. (2005) observed that low Se concentrations increased chlorophyll content in soybean plants, corroborating the results of this study. In addition, Silva et al. (2020) observed that Se applied as sodium selenate at 2.5 g ha^{-1}, a lower concentration, produced a notable increase in chlorophyll a,

chlorophyll b, total chlorophyll, and carotenoid concentrations in cowpea.

In general, the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) evaluated in this study were not sensitive to Se. Similar results were observed by Ramos et al. (2011) in lettuce germplasm, in which APX, CAT, and glutathione peroxidase (GSH-Px) activities showed no significant differences in response to selenate and selenite treatments. Moreover, Lara et al. (2019) evaluated wheat grain biofortification via foliar application of Se and found that Se increased APX activity, but CAT and SOD showed no sensitivity to Se.

Regression curves were presented only for variables with a significant fit to the polynomial model, namely root length and Se content in dry mass. The regression coefficients for the remaining variables were not statistically significant ($p > 0.05$), indicating the absence of a dose–response relationship. Therefore, graphical representation through regression models was not biologically or statistically justified for these variables.

Root length had a significant quadratic response to sodium selenate concentrations, with an initial increase and a maximum point estimated at approximately 11.49 µmol L^{-1}, followed by a reduction at higher concentrations (Figure 7).

In contrast, Se content in dry mass showed an increasing trend, fitted the polynomial model, and indicated progressive Se accumulation in plant tissues as a function of the applied concentration (Figure 8).

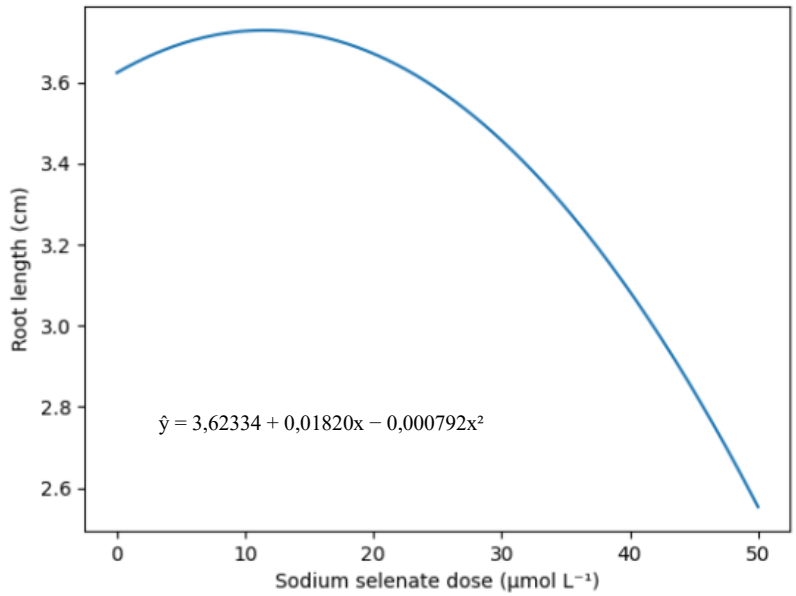

Figure 7. Quadratic regression of root length of 'Prata Anã' Gorutuba banana plantlets as a function of sodium selenate concentrations.

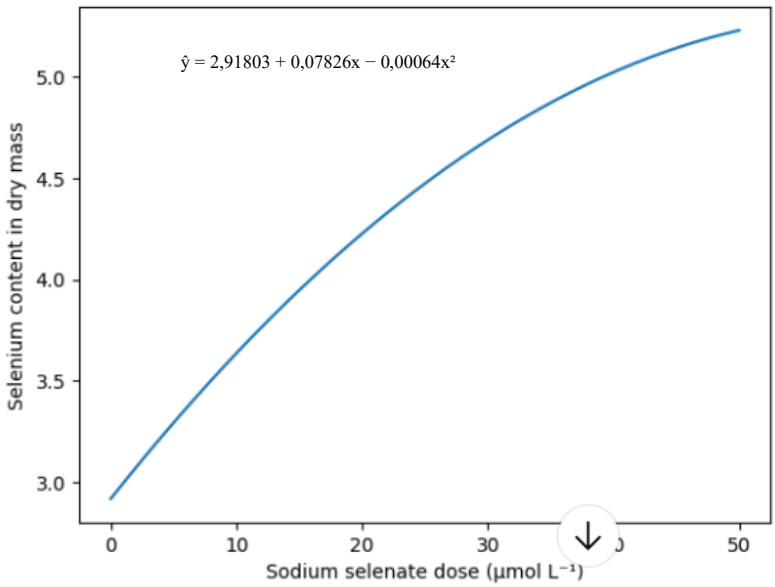

Figure 8. Polynomial regression of Se content in dry mass of 'Prata Anã' Gorutuba banana plantlets as a function of sodium selenate concentrations.

4. Conclusions

The application of 20 µmol L^{-1} sodium selenate was the most suitable concentration for Se biofortification of micropropagated 'Prata Anã' Gorutuba banana plantlets, promoting significant Se accumulation without impairing growth or photosynthetic performance.

Higher Se concentrations increased Se accumulation but reduced plant growth, indicating a dose-dependent toxic effect at high concentrations. During acclimatization, intermediate concentrations maintained a better balance between biofortification efficiency and plantlet quality.

Photosynthetic pigments responded positively to lower Se concentrations, whereas antioxidant enzyme activity was not significantly altered. Overall, Se biofortification via micropropagation is feasible when appropriate concentrations are used to maintain plant quality and physiological stability.

Authors' Contribution

Martha Cristina Pereira Ramos: Conceptualization, methodology, investigation, data collection, data analysis, interpretation of results, scientific writing, and manuscript preparation.

Renata Amato Moreira: Investigation, experimental execution, data collection, and contribution to data analysis and interpretation.

Mariane Aparecida Rodrigues: Experimental execution, investigation, data collection, and contribution to data analysis and interpretation.

Leila Aparecida Salles Pio: Conceptualization, supervision, project administration, methodology development, scientific guidance, critical review, and editing of the manuscript.

Julio Sílvio de Sousa Bueno Filho: Experimental design, statistical methodology, data analysis, statistical interpretation, and critical review of the manuscript.

Ana Claudia Costa Baratti: Contribution to data analysis and interpretation, scientific writing, manuscript review, editing, and intellectual contributions to the study.

Acknowledgments

The authors gratefully acknowledge the financial support provided by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) through scholarships and research funding that contributed to the development of this study.

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