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Research Article

# Influence of basal medium formulations and silver nanoparticles on in vitro plant growth in gerbera

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**Abstract** This study investigated the impact of two distinct MS basal media: one containing FeNaEDTA and the other FeEDDHA, on the growth of five unique gerbera cultivars (Shy Pink, Pink Holic, Breeze, Harmony, Snow Dream). Notably, the response to these media types varied significantly among the cultivars, particularly concerning leaf yellowing and plant growth. 'Shiny Pink' and 'Pink Holic' exhibited leaf yellowing on the FeNaEDTA-containing medium but displayed leaf greening on the FeEDDHA-containing medium. In contrast, 'Snow Dream,' 'Harmony,' and 'Breeze' remained unaffected on both medium types. However, the FeNaEDTA-containing medium promoted higher plant height and petiole length in 'Breeze,' 'Harmony,' and 'Snow Dream' than the FeNaEDTA-containing medium did. A promotive effect of silver nanoparticles (AgNPs) on plant growth and leaf greening was observed in 'Pink Holic,' particularly on the FeNaEDTA-containing medium, while the addition of AgNPs to the FeEDDHA-containing medium negatively affected plant growth. These results highlight the substantial influence of medium type, specifically the presence of FeNaEDTA or FeEDDHA, on gerbera growth responses, emphasizing the critical role of medium selection in gerbera propagation. Additionally, when contemplating the addition of AgNPs for in vitro gerbera propagation, it is crucial to consider the medium type.

**Keywords** Leaf yellowing, MS medium types, sliver nanoparticles, Plant growth parameters, SPAD values

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## Introduction

Gerberas are extensively used as cut flowers worldwide, maintaining their position among the top 10 most popular cut flowers globally (Ahmed et al. 2018). The production value of cut gerbera reached approximately 36 million USD in 2016 (Deng and Bhattarai 2018). In vitro plant propagation techniques have been widely employed for commercial production of economically important gerbera plant species (Bhatia et al. 2011; Gantait and Mahanta, 2022; Mahanta et al. 2023; Mosqueda Frómeta et al. 2017; Tung et al. 2022b). However, previous studies have reported abnormalities and leaf yellowing in the regenerated plants, and these symptoms persisted even when the plants were subcultured on Murashige and Skoog (MS) basal medium, which contains FeNaEDTA, to promote plant growth and rooting. Therefore, addressing these abnormalities and leaf yellowing is imperative for ensuring the commercial production of quality gerbera plantlets.

Zawadzka and Orlikowska (2006) reported that ethylene-diamine di-2-hydroxy-phenyl acetate ferric (FeEDDHA) reduced chlorosis and increased chlorophyll contents in in vitro regenerated shoots of red raspberry cultivars. Similarly, Licea-Moreno et al. (2015) also observed that substituting FeEDTA with FeEDDHA diminished chlorotic symptoms and significantly improved the rooting ability of walnuts. Additionally, hazelnut shoots were observed to have longer

and a higher number of nodes when cultured on a medium supplemented with higher concentration of FeEDDHA (Garrison et al. 2013). Moreover, Al-Mayahi et al. (2021) also reported a positive effect of FeEDDHA on increasing chlorophyll contents in regenerated date palm plants. In our preliminary work, when four-week-old in vitro plants of two different gerbera cultivars were cultured on MS basal medium containing FeNaEDTA, they exhibited leaf yellowing and low chlorophyll contents. Therefore, it was interesting to investigate the influences of two MS medium types, each containing FeNaEDTA or FeEDDHA, in plant growth and leaf yellowing in the gerbera cultivars.

Recently, silver nanoparticles (AgNPs) has been reported to have a promotive effect on in vitro plantlet growth in chrysanthemum (Tung et al. 2021a), strawberries (Tung et al. 2021b), Anthurium (Tung et al. 2022a), gerbera (Tung et al. 2022b), and hosta (Pe et al. 2020), whereas AgNPs not only promoted shoot growth but also reduced leaf yellowing of the shoots, ensuring the production of high-quality shoots. It was interesting to test the effects of AgNPs as well on reducing leaf yellowing and improving chlorophyll contents for the commercial production of high-quality plants.

Therefore, in this study, we investigated the influences of two MS medium types, one containing FeNaEDTA and the other FeEDDHA, on plant growth and leaf yellowing in gerbera cultivars. Additionally, we examined the effect of AgNPs on the same plant growth and leaf yellowing in gerbera 'Pink Holic'.

## Materials and Methods

### Plant materials

Four-week-old in vitro plants of five different gerbera cultivars (Shy Pink, Pink Holic, Breeze, Harmony, Snow Dream), obtained from Gana Flower Seedling company (Hadong, Korea), were used as plant materials in this study.

### Effects of Murashige and Skoog (MS) medium types on in vitro plant growth of the gerberas

Two different types of Murashige and Skoog (MS) media were used in this study. The compositions of the two MS media are identical, except for the presence of FeNaEDTA or FeEDDHA. One MS medium contains FeNaEDTA, while the other substitutes FeNaEDTA with FeEDDHA. Four-week-old in vitro plants of gerberas (cvs. Shy Pink, Pink Holic, Breeze, Harmony, and Snow Dream), obtained from Gana Flower Seedling Company, were cultured on the two different media. These media were supplemented with 3.0% sucrose and 8.0 g/L agar (Duchefa Biochemie, the Netherlands). The pH of the media was adjusted to 5.8 before autoclaving at 121°C for 16 min. Only uniform plant sizes were selected for this investigation. Each bottle contained two plants, and there were five bottles per treatment, with three replications. All bottles were placed in a culture room set at a temperature at 25 ± 2°C under 16 h photoperiod with a light intensity of 77.3 μmol $m^{-2}$ $s^{-1}$. After four weeks of culture, the plant growth parameters (plant height, leaf size, and petiole length) and SPAD values were assessed in the gerberas. Each measurement contains ten plants, with three replications.

### Measurement of SPAD values

SPAD values were measured in the leaves of in vitro plants derived from the two different medium types using a chlorophyll meter (SPAD-502, Minolta, Japan). Each measurement contains ten plants, with three replications.

### Effects of silver nanoparticles (AgNPs) on plant growth of cv. Pink Holic

Four-week-old in vitro plants of cv. Pink Holic were cultured on the aforementioned two different MS media, each supplemented with different concentrations of AgNPs (0, 1.0, 1.5, and 2.0 mg/L). Each bottle contained two plants, and there were five bottles per treatment, with three replications. All bottles were placed in a culture room set at a temperature at 25 ± 2°C under 16 h photoperiod with a light intensity of 77.3 μmol $m^{-2}$ $s^{-1}$. After four weeks of culture, the plant growth parameters (plant height, leaf size, and petiole length) and SPAD values were assessed in the gerberas. Each measurement included ten plants, with three replications. SPAD values were measured as described above.

### Statistical analysis

Data were analyzed using SPSS version 11.09 (IBM Corporation, Armonk, NY, United States) and expressed as mean (of three replicates) ± standard error. To distinguish between mean values, the Student t-test was employed, with the levels of significance set at $p < 0.01$ and 0.05.

## Results

### Effects of two different MS basal medium types on plant growth of the gerberas

Four-week-old in vitro plants of five different gerbera cultivars (Shy Pink, Pink Holic, Breeze, Harmony, Snow Dream) were cultured on two types of MS medium, each containing either FeNaEDTA or FeEDDHA. After four weeks of culture, we observed the differential growth responses among the gerberas based on the presence of FeNaEDTA or FeEDDHA in the MS medium. Cultivars 'Shy Pink' and 'Pink Holic' exhibited leaf yellowing on the MS medium containing FeNaEDTA, while the yellowing symptoms were not observed on the medium containing FeEDDHA (Fig. 1). When measuring SPAD values in the leaves, values were significantly lower in the plants derived from the medium containing FeNaEDTA compared to those in the plants derived from the medium containing FeEDDHA (Fig. 2), indicating an association between the reduction of SPAD value and leaf yellowing. In contrast, plant growth parameters such as plant height and leaf size did not significantly differ between the two types of medium for both cultivars (Fig. 2). However, the petiole length of cv. Pink Holic was longer in the FeEDDHA-containing medium than in the FeNaEDTA-containing medium. This difference in petiole length between the two medium types was not observed in cv. Shy Pink (Fig. 2).

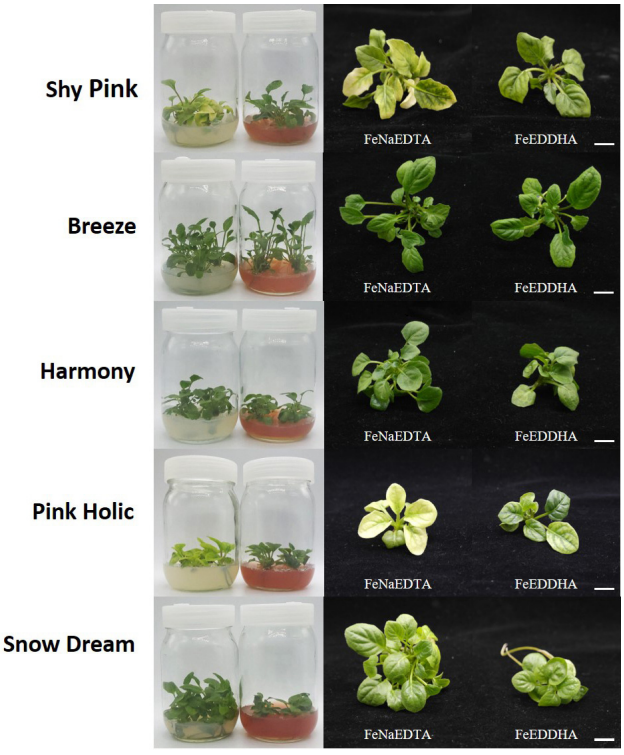


**Fig. 1** Illustration of growth status and leaf color (yellowing or greening) of different gerbera cultivars cultured on two different types of MS media, each containing FeNaEDTA or FeEDDHA. These images were taken after four weeks of culture

Leaf yellowing did not occur in the other cultivars 'Snow Dream', 'Harmony', and 'Breeze' when they were cultured on the two medium types (Fig. 1). This was corroborated by the results of SPAD values, as there were no significant differences in values between the two medium types for these cultivars (Fig. 2). Nevertheless, in the case of cv. Breeze, FeNaEDTA-containing medium led to improved plant height and petiole length compared to the FeEDDHA-containing medium. Similarly, the FeNaEDTA-containing medium resulted in greater plant heights for the 'Harmony' and 'Snow Dream' cultivars than the FeEDDHA-containing medium did (Fig. 2). However, leaf sizes of the cvs. 'Harmony' and 'Breeze' showed no difference between the two medium types, even though there was a longer petiole length in cv. Breeze on FeNaEDTA-containing medium. Surprisingly, in cv. 'Snow Dream', leaf size and petiole length observed in the FeNaEDTA-containing medium were notably greater than those in the FeEDDHA-containing medium (Fig. 1 and 2).

## Effect of different concentrations of AgNPs on plant growth of cv. Pink Holic

Four-week-old in vitro plants of cv. Pink Holic were cultured on two MS medium types, with each medium containing either FeNaEDTA or FeEDDHA. Both of these mediums were supplemented with different concentrations of AgNPs (0, 1.0, 1.5, and 2.0 mg/L) to access the effect of AgNPs on plant growth of the gerbera. As observed above, cv. Pink Holic showed leaf yellowing when cultured on the FeNaEDTA-containing medium (AgNPs; 0 mg/L), a phenomenon not observed in the FeEDDHA-containing medium (AgNPs; 0 mg/L) (Fig. 3). The addition of AgNPs (at concentrations ranging from 1.0 to 2.0 mg/L) to the FeNaEDTA-containing medium notably exhibited a greener appurtenance of the plants, distinct from those grown on the AgNPs -free medium (0 mg/L) (Fig. 3). However, AgNPs addition to the FeEDDHA-containing medium had no significant impact on the plants' green appearance. This aligns with the results of SPAD values, where values in the plants derived from the FeNaEDTA-containing medium with AgNPs were significantly higher than those in the plants from the medium without AgNPs. Additionally, the values did not significantly differ between the plants cultured in the FeEDDHA-containing medium, with or without AgNPs (Fig. 3).

When examining plant growth parameters such as plant height, leaf size, and petiole length, the presence or absence of AgNPs in the FeNaEDTA-containing medium

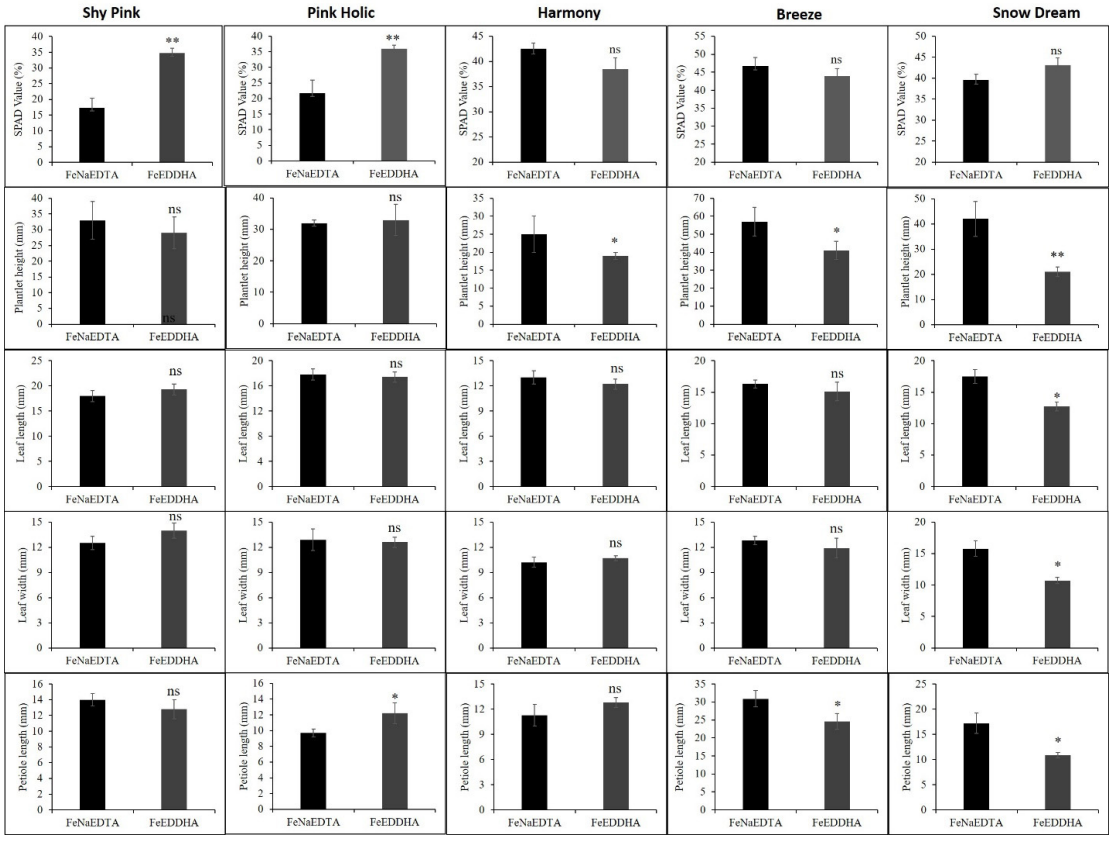


**Fig. 2** Illustration of SPAD values and plant growth parameters (plant height, leaf length, leaf width, and petiole length) of different gerbera cultivars cultured on two different types of MS media, each containing FeNaEDTA or FeEDDHA. These data were taken after four weeks of culture. Data represent the mean values of three replicates, and error bars indicate the standard error. Means with asterisk(s) are statistically significant (T-test, **p < 0.01, *p < 0.05). ns= non-significant

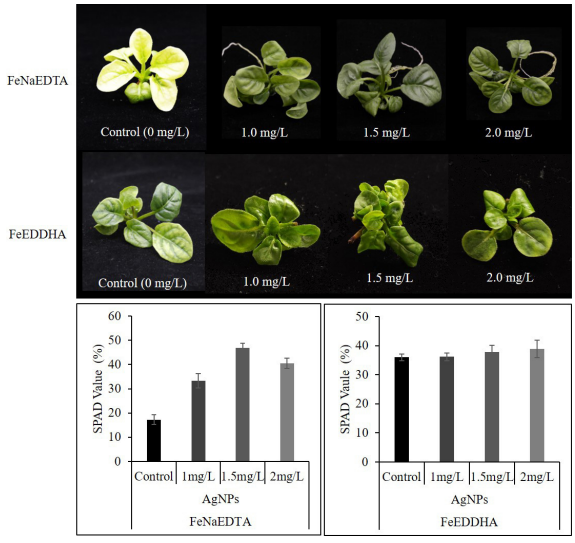


**Fig. 3** Illustration of plant growth status, leaf color (yellowing or greening), and SPAD values of gerbera 'Pink Holic' cultured on two different types of MS media, each containing FeNaEDTA or FeEDDHA. These media were supplemented with varying concentrations of AgNPs (0, 1, 1.5, and 2 mg/L). These images were taken after four weeks of culture. Data represent the mean values of three replicates, and error bars indicate the standard error

did not significantly affect these growth parameters (Fig. 4). However, in the case of the FeEDDHA-containing medium, the addition of AgNPs led to a reduction in the growth parameters (Fig. 4).

## Discussion

Previous studies experienced abnormalities and leaf yellowing in the regenerated plants of gerberas when explants were cultured on the most commonly used MS basal medium containing FeNaEDTA (Bhatia et al. 2011; Gantait and Mahanta, 2022; Mahanta et al. 2023; Mosqueda Frómeta et al. 2017; Tung et al. 2022b). In an effort to address leaf yellowing, another MS medium containing FeEDDHA has been employed for in vitro regeneration in several plant species (Al-Mayahi et al. 2021; Garrison et al. 2013; Licea -Moreno et al. 2015; Zawadzka and Orlikowska, 2006). However, it remains unknown whether the MS medium containing FeEDDHA can effectively reduce leaf yellowing in regenerated gerbera plants. Therefore, in this study, four-week-old gerbera plants were cultured on two different

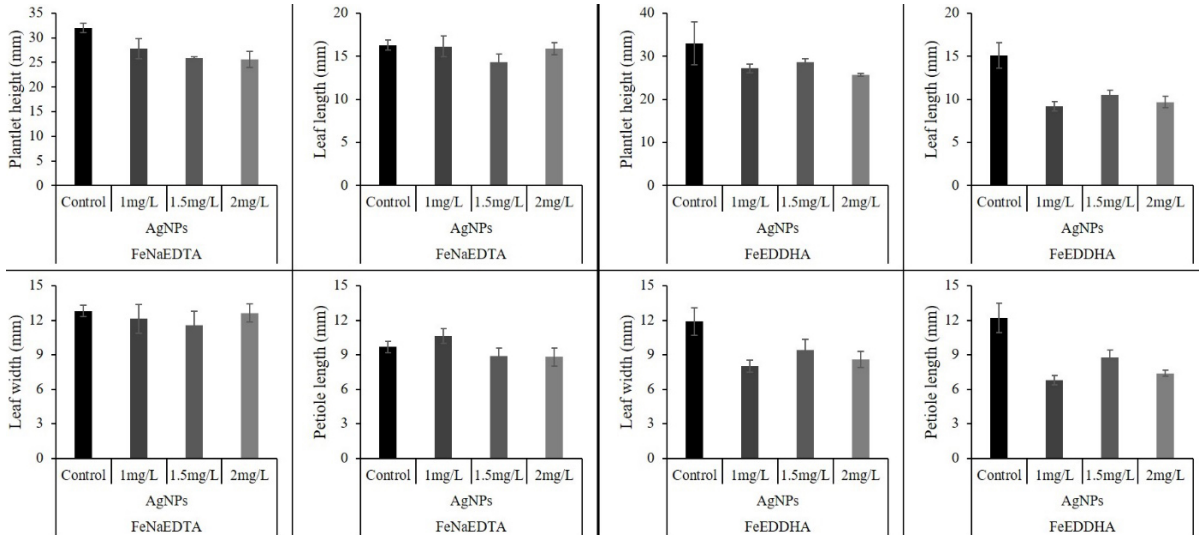


**Fig. 4** Illustration of plant growth parameters (plant height, leaf length, leaf width, and petiole length) of gerbera 'Pink Holic' cultured on two different types of MS media, each containing FeNaEDTA or FeEDDHA. These media were supplemented with varying concentrations of AgNPs (0, 1, 1.5, and 2 mg/L). These data were taken after four weeks of culture. Data represent the mean values of three replicates, and error bars indicate the standard error

types of MS medium, each containing either FeEDDHA or FeNaEDTA.

We observed that the two different types of MS medium responded differently to the in vitro plant growth of the gerberas. The differential effects of the medium types on plant growth and yellowing were confirmed by analyzing several plant growth parameters and SPAD values. The medium containing FeNaEDTA exhibited improved plant growth in the cultivars 'Breeze', 'Harmony', and 'Snow Dream' compared to those cultured on the medium containing FeEDDHA. However, the cultivars 'Shy Pink' and 'Pink Holic' cultured on the same medium displayed more yellowing in their leaves than those cultured on the medium containing FeEDDHA. It appears that presence of FeNaEDTA in the MS medium is more suitable for the growth of the cultivars 'Breeze', 'Harmony', and 'Snow Dream,' but not for the cultivars 'Shy Pink' and 'Pink Holic.' Conversely, the presence of FeEDDHA in the MS medium is more appropriate for the cultivars 'Shy Pink' and 'Pink Holic' rather than the other cultivars. This indicates the necessity of the screening the MS medium type for specific gerbera cultivars. The occurrence of abnormalities and leaf yellowing in regenerated gerbera plants was observed on the MS basal medium containing FeNaEDTA (Bhatia et al. 2011; Gantait and Mahanta, 2022; Mahanta et al. 2023; Mosqueda Frómeta et al. 2017; Tung et al. 2022b). It was likely that the gerberas used in the previous studies were genetically similar to the cultivars 'Shy Pink' and 'Pink Holic', as these cultivars also displayed leaf yellowing on the MS medium containing FeNaEDTA.

The effects of FeEDDHA on the reduction of chlorosis and the improvement of chlorophyll contents in in vitro regenerated shoots have been observed in red raspberry, walnuts, hazelnut, and dates (Al-Mayahi et al. 2021; Garrison et al. 2013; Licea-Moreno et al. 2015; Zawadzka and Orlikowska, 2006). We also observed the promotive effect of FeEDDHA on SPAD values and leaf greening in the cultivars' Shy Pink' and 'Pink Holic', which exhibited distinct leaf yellowing on the MS medium containing FeNaEDTA. However, no promotive effect of FeEDDHA on SPAD values was observed in the other cultivars 'Breeze', 'Harmony', and 'Snow Dream,' compared to those cultured on the MS medium containing FeEDDHA. This can be explained by the fact that these cultivars grew well on the MS medium containing FeNaEDTA, resulting in high SPAD values, and therefore, their SPAD values were similar to those cultured on the MS medium containing FeEDDHA.

The addition of AgNPs to the MS medium containing FeNaEDTA apparently showed distinct greening of leaves in the 'Pink Holic' compared to those cultured on the same medium without AgNPs. The involvement of AgNPs in chlorophyll biosynthesis has also been noted in *Vigna radiata* and *Vanilla planifolia*. In these plants, it led to a significant increase in leaf chlorophyll content and enhanced plant fresh weight (Saeideh and Rashid 2014; Spinoso-Castillo et al. 2017). Furthermore, AgNPs has demonstrated a positive impact on in vitro plantlet growth in various species, including chrysanthemum (Tung et al. 2021a), strawberries (Tung et al. 2021b), Anthurium (Tung et al. 2022a), and hosta (Pe et al. 2020). Notably, in addition to

promoting shoot growth, AgNPs has been found to mitigate leaf yellowing in the shoots. In our study, we found that 1.5 mg/L AgNPs was the best for leaf greening, as the SPAD value observed at 2.0 mg/L was lower than that at 1.5 mg/L. However, in a study done by Tung et al. (2022b), AgNPs at a concentration of 2.0 mg/L was deemed optimal for shoot regeneration and the reduction of leaf yellowing in gerbera. Concentrations of AgNPs higher than 2.0 mg/L led to a reduction in plant growth and SPAD values. The differences in the optimal AgNPs concentrations between the two studies may be attributed to variations in the stages of in vitro plants and the genotypes used. Jadczak et al. (2019) and Pe et al. (2020) also observed that higher concentrations of AgNPs led to a reduction in plant growth, and the optimal AgNPs concentration required for plant growth varied depending on the sizes of nanoparticles and genotypes used. Surprisingly, the inclusion of AgNPs in the MS medium containing FeEDDHA did not improve SPAD values; instead, it had a negative impact on plant growth parameters. It appeared that the MS medium containing FeEDDHA does not benefit from addition of NAgP, while the MS medium containing FeNaEDTA requires AgNPs to promote plant growth and reduce leaf yellowing. Therefore, this study suggests that when considering the addition of AgNPs for plant growth and leaf greening, it is necessary to consider the types of MS medium, as the promotive effect of AgNPs on plant growth and leaf greening depends on the presence of either FeNaEDTA or FeEDDHA.

## Conclusion

The differential effects of the medium types on plant growth and yellowing were in different gerberas, as confirmed by the analysis of several plant growth parameters and SPAD values. The medium containing FeNaEDTA exhibited improved plant growth in the cultivars ‘Breeze’, ‘Harmony’, and ‘Snow Dream’ but led to leaf yellowing in ‘Shy Pink’ and ‘Pink Holic’. Conversely, the presence of FeEDDHA in the MS medium was more appropriate for ‘Shy Pink’ and ‘Pink Holic’ rather than for ‘Breeze’, ‘Harmony’, and ‘Snow Dream’. Additionally, we observed a promotive effect of AgNPs on leaf greening in ‘Pink Holic,’ particularly when it was added to the MS medium containing FeNaEDTA only. This study underscores the importance of screening the appropriate MS medium type for specific gerbera cultivars. Moreover, when considering the addition of AgNPs for in vitro propagation of gerberas, it is crucial to take the medium type into account.

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