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Boosting lily bulblets production: a study on the effects of 6-benzylaminopurine and silver nanoparticles

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The lily stands out as one of the most economically relevant flowers worldwide and enjoys great popularity as a cut flower in the florist industry. This prominent position underlines the need to implement and continuously improve sustainable protocols that ensure its efficient production, multiplication and conservation. In this context, the present study aimed to investigate the potential biostimulant effect of Silver Nanoparticles (AgNPs) and 6-Benzylaminopurine (BAP) on two lily cultivars (*Lilium* LA Hybrid 'Hyde Park' and *Lilium* LA Hybrid 'Yellow Diamond'), through their individual and combined application. The research focused on evaluating the influence of these treatments on two key aspects: (1) regeneration capacity and bulblet development from bulb scales, and (2) morpho-physiological growth parameters in regenerated plants. Analysis of the results revealed several significant effects of the interaction between AgNPs and BAP on different regeneration and growth parameters in two lily cultivars. In cv. 'Hyde Park', the combination of AgNPs at 50 mg L^{-1} with BAP at 200 mg L^{-1} increased bulblet number to an average of 5.6. In cv. 'Yellow Diamond', the application of AgNPs at 50 mg L^{-1} without BAP and the combined treatment of AgNPs at 100 mg L^{-1} with BAP at 200 mg L^{-1} produced a higher number of bulblets (3.2). The highest bulblet weight in both cultivars was obtained with the combination of AgNPs at 100 mg L^{-1} and BAP at 50 mg L^{-1}. Plant height also varied considerably; in cv. 'Hyde Park' the tallest plants (greater than 140 mm) were recorded under treatment with BAP at 50 mg L^{-1} without AgNPs, while in cv. 'Yellow Diamond', the largest plants (greater than 100 mm) were observed with the application of AgNPs at 100 mg L^{-1} without BAP. In terms of physiology, the SPAD index did not show significant improvements compared to the control group. In conclusion, the results indicate that the impact of the interaction between AgNPs and BAP is concentration, cultivar, and measured variable dependent, suggesting a varietal-specific response and the possibility of both beneficial and adverse effects on different aspects of lily development. This underscores the importance of carefully adjusting doses and combinations to optimize regeneration and plant growth.

Keywords Bulb scale, Cytokinin, Growth regulators, Nanotechnology, Propagation

Liliaceae are a plant family over 3000 species and 250 genera[1] and, their increasing popularity is largely attributed to the introduction of new cultivars that produce larger, more attractive and fragrant flowers[2].

Ornamental flower production constitutes approximately 50% of the horticultural industry, with bulbous flowers, such as lilies (*Lilium* sp., Liliaceae) being particularly popular[2]. Peru's geographic location and varied climatic conditions enable the cultivation of lilies across a wide range of altitudes. In regions such as Amazonas (Chachapoyas) and Ancash (Caraz), they are successfully cultivated[3,4]. This provides an opportunity for rural

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areas to diversify their agricultural production. However, large scale production remains limited in countries with developing economies due to the scarcity and high import costs of bulbs (U$S 168,000.53 for 570,000 bulbs in 2021)[5].

This scenario raises the need to explore new methods to produce high quality bulbs, and that above all benefit the small producer[6]. Traditionally, the method of obtaining propagules consists of harvesting bulbs developed during the previous season, being a process that takes place in the leaf axils, stem and scales[4]. The latter contains a large amount of reserve nutrients and also harbor in their axils meristems that can give rise to miniature bulbs "bulbils"[3]. However, achieving commercial viability requires extended periods (up to 36 months), during which bulb health and vigor critical for market success are not guaranteed[7].

Under this perspective, nanotechnology has offered diverse applications in agriculture, including the development of plant protection products[8], herbicides[9], fertilizers[10] and, solutions to extend the shelf life of cut flowers[11]. They have also been reported to exhibit biostimulant and biocidal properties[12]. In particular, silver nanoparticles (AgNPs) have demonstrated efficacy in promoting shoot induction[13], enhancing biomass accumulation[14], stimulating seed germination[15], and improving tolerance to heavy metals[16]. However, it is crucial to consider the risks associated with their use, as high concentrations can be detrimental to photosynthesis, flowering and growth[17]. Despite this, their use in agronomy is justified by the hypothesis that their effects are highly dependent on dose, particle size, type of coating and environmental conditions[18]. Consequently, further research is required to elucidate the dualistic effects of AgNPs on plant systems, with the aim of expanding their application in agriculture.

The application of AgNPs in floriculture has been little explored, recently Salachna et al.[2] highlighted their potential potential to stimulate growth and flowering of lily cultivars 'Mona Lisa' and 'Little John'. Similarly, Byczyńska et al.[19] reported that AgNPs at concentrations ≤ 150 mg L^{-1} increased bulblet number and fresh weight in *Lilium* cv. 'Osasco'. These findings underline the need for careful evaluations to mitigate risks of phytotoxicity or antagonistic interactions.

On the other hand, biostimulants and plant growth regulators (PGRs) are compounds that modulate plant cell development. Nonetheless, it has been repeatedly stated that it is important to determine the dose and combination of these substances to validate their efficacy on plant regeneration and multiplication capacity. In particular, 6-benzylaminopurine (BAP), an exogenous cytokinin, has been shown to significantly stimulate shoot proliferation in ornamental geophytes[5]. For example, Vera et al.[4] reported that specific concentrations of BAP, such as 100 mg L^{-1}, can significantly increase *Lilium* bulblets formation. Nevertheless, it is essential to note that the optimal dose may vary depending on plant species and cultivation conditions. In this regard, Ren et al.[20] demonstrated that lower BAP concentrations, such as 4 mg L^{-1}, may be more effective in *Lycoris sprengeri*.

The integrated use of BAP and AgNPs could trigger a synergistic effect of considerable value in horticulture. BAP exerts direct action on shoot cell division and proliferation, while AgNPs can influence physiological processes associated with stress tolerance and promote tissue regeneration[16]. In lily production, this synergy could manifest itself in a substantial improvement of bulblet regeneration through complementary mechanisms, such as activation of genes involved in meristematic development, increased antioxidant capacity and modulation of endogenous phytohormones[21]. However, further research is needed to fully understand their combined effects.

Therefore, with the hypothesis that the application of silver nanoparticles (AgNPs) and 6-benzylaminopurine (BAP) could generate a biostimulant effect, the present study focused on investigating the potential of these compounds, both individually and in combination, in lily cultivation. Specifically, the research aimed to evaluate the influence of AgNPs and BAP on two key aspects: (1) the regeneration capacity and the development of bulblets from bulb scales, and (2) the morpho-physiological growth of the regenerated plants.

Materials and methods

Study area

The research was conducted out in the nursery of the experimental station of the Research Institute for Sustainable Development of Ceja de Selva, affiliated at the Universidad Nacional Toribio Rodríguez de Mendoza de Amazonas (coordinates: 6°23′39 " S; 77°85′20" W).

Plant material

Bulbs of *Lilium* LA Hybrid 'Yellow Diamond' and *Lilium* LA Hybrid 'Hyde Park' were collected from production fields located in the Taquia Annex, Chachapoyas province (Coordinates: 6° 15′ 28.1″ S; 77° 49′ 52.7″ W). The bulbs selected for the study met strict criteria, including a minimum weight of 30 g and an equatorial diameter greater than 7 cm. A permit for scientific research on wild flora (RDG N° D000394-2020-MIDAGRI-SERFOR-DGGSPFFS, with authorization code N° AUT-IFL-2020–061) was provided by Servicio Nacional Forestal y de Fauna Silvestre (SERFOR).

Cleaning and separation of scales

Bulbs were cleaned under running water and disinfected with a 1.5% (v/v) sodium hypochlorite solution for 10 min, followed by a benomyl solution (50 WP) at 1 mg L^{-1} for 20 min. Subsequently, the external scales were removed, and internal scales 3 to 4 cm length and 2 to 3 cm width were extracted.

Growing conditions

Scale propagation was conducted in 32-cell culture trays filled with a sterile substrate comprising *Sphagnum* moss blonde peat and vermiculite (PREMIX #3, Agriplant). Previously, solutions of BAP (0, 50, and 200 mg L^{-1}) and AgNPs (0, 50, and 100 mg L^{-1}) were prepared in ethyl alcohol and ultrapure water, respectively. BAP purchased from Sigma-Aldrich (Product number B3408) was used. In addition, AgNPs obtained from SkySpring Nanomaterials (Product number 0120XH), characterized by a purity of 99.95%, particle size of 20–30 nm, spherical shape and a specific surface area of ~ 20 m^2/g, were used. The scales were immersed for 60 min in the solutions according to treatment and then seeded individually in the cells of the trays with the substrate moistened.

After 45 days, the initial evaluation quantified bulblet regeneration efficiency by recording the number of bulblets per scale. Regenerated bulblets were carefully extracted and planted individually in 5 " × 8" nursery bags containing the above-mentioned substrate. Plants were grown under natural photoperiod conditions in a tunnel covered with raschel mesh with 50% shade (average photon flux density: 1052.75 µmol m^2 s^{-1}). During the experiment the average temperature was 17 °C and average humidity was 80%. Plant development was determined after 90 days.

Morphological indicators evaluated

Once the experiment was established, the number of regenerated micro bulbs was determined after 45 days. In the following 90 days, bulb weight, bulb equatorial and polar diameter, number of leaves and roots, root length, plant height and leaf area were evaluated. The evaluations were carried out with a digital vernier, and the leaf area was determined by means of the Software imagen J (https://imagej.net/ij/index.html).

Physiological indicators evaluated

The relative chlorophyll content (SPAD index) was determined after 90 days, using a SPAD-502 Chlorophyllometer (Konica Minolta, Tokyo, Japan). Readings were taken between 10:00 a.m. and 1:00 p.m., on three leaves per plant, and the average was recorded at the end of each day.

Stomatal conductance (mmol/m^2 s) was determined after 90 days, using a SC-1 leaf porometer (Decagon Devices, Pullman WA, USA). Readings were taken between 10:00 a.m. and 1:00 p.m., on three leaves per plant and the average was recorded.

Experimental design and statistical analysis

The lily cultivars were set up in two separate experiments, under a completely randomized design with a 3 × 3 bifactorial arrangement (AgNPs dose x BAP dose), giving rise to 9 treatments. The experimental unit for the evaluation of bulblet induction was the scale, with 10 replicates per treatment. For the evaluation of seedling development, the experimental unit was the bulblet, with 10 replicates per treatment. The data were adjusted to a normal distribution and were subjected to an analysis of variance (ANOVA) and the means were compared using Tukey's test ($p < 0.05$). Additionally, a Pearson's correlation test was performed. All processing was performed using the R software v. 4.4.2.

Results

Morphological indicators

Number of bulblets

The result of the two-way analysis of variance (ANOVA) revealed a significant effect of the AgNPs × BAP interaction on the number of regenerated bulblets for both cv. LA Hybrid 'Hyde Park' ($F = 6.74$; $p < 0.001$) and cv. LA Hybrid 'Yellow Diamond' ($F = 3.24$; $p < 0.05$). The results presented in Fig. 1 (left side) show that, in cv. 'Hyde Park', the application of AgNPs at 50 mg L^{-1} in the presence of the highest concentration of BAP (200 mg L^{-1}) increased the number of regenerated bulblets to an average of 5.6 ± 0.89 bulblets per scale. Despite this, it is found that the highest concentration of BAP tends to inhibit regeneration in this cultivar, and while the addition of AgNPs does not completely reverse this inhibition, it does suggest a slight trend toward improvement. For cv. 'Yellow Diamond' (Fig. 1, right side), the results indicate that the number of bulblets did not differ significantly among the different BAP concentrations (0, 50, 200 mg L^{-1}) when AgNPs were not applied. In contrast, the application of AgNPs at 50 mg L^{-1} (without BAP) and at 100 mg L^{-1} together 200 mg L^{-1} of BAP led to a significantly higher number of bulblets per scale (3.2 units on average) compared to the control treatment. Overall, the comparison between cultivars revealed that 'Hyde Park' produced significantly more bulblets than 'Yellow Diamond'. This result supports the idea that the response to AgNPs and BAP application in bulblet regeneration exhibits varietal specificity.

At 45 days post-experiment initiation, Fig. 2 illustrates the differential response of bulb scales to AgNPs and BAP treatments on bulblet formation. It was observed that the treatments not only influenced the number of bulblets, but also their development; for example, in the cv. 'Hyde Park' (Fig. 2a), treatment with AgNPs and BAP at optimal concentrations induced accelerated bulblet development, manifested in early leaf formation. However, this effect was less evident in the cv. 'Yellow Diamond' (Fig. 2b). These results indicate that the combination of AgNPs and BAP could be a valuable tool to improve vegetative propagation in some lily cultivars, although optimization of concentrations is essential to achieve optimal results.

Polar and equatorial bulblet diameter

Two-way analysis of variance (ANOVA) revealed a significant interaction effect between AgNPs and BAP on the polar diameter of regenerated bulblets in both cultivars studied: LA Hybrid 'Hyde Park' ($F = 5.70$; $p < 0.05$) and LA Hybrid 'Yellow Diamond' ($F = 6.38$; $p < 0.05$). The results indicate that the largest polar diameter was in control treatment for both 'Hyde Park' (23.90 mm, left side of Fig. 3a) and 'Yellow diamond' (21.18 mm, right side of Fig. 3b), suggesting possible adverse effects of the experimental treatments on this variable.

On the other hand, Fig. 3b presents the results of the equatorial diameter of the regenerated bulblets. Analysis of the results obtained in cv. 'Hyde Park' ($F = 4.01$; $p < 0.01$) as in cv. 'Yellow Diamond' ($F = 5.40$; $p < 0.001$) shows that the application of AgNPs without addition of BAP seems to have a negative effect on the equatorial diameter. However, it is interesting to note that this trend seems to be partially reversed when combining AgNPs with BAP at 50 mg L^{-1}. These results underscore the importance of continuing research to elucidate the mechanisms

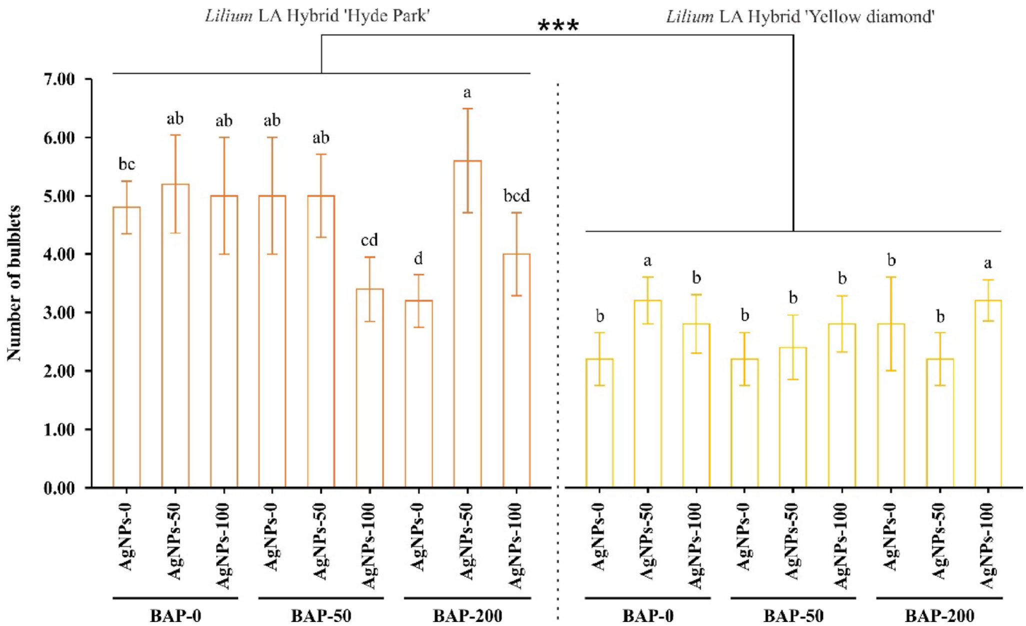

Fig. 1. Number of regenerated bulblets by two *Lilium* hybrids (LA Hybrid 'Hyde Park' and LA Hybrid 'Yellow diamond') treated with different concentrations of AgNPs and BAP. Data were analyzed with a Two-way ANOVA followed by Tukey's multiple comparison test. Values are presented as the mean ± Standard deviation. Different letters indicate a significant difference at $p < 0.05$. Asterisks indicate significant differences between cultivars (*** $p < 0.001$).

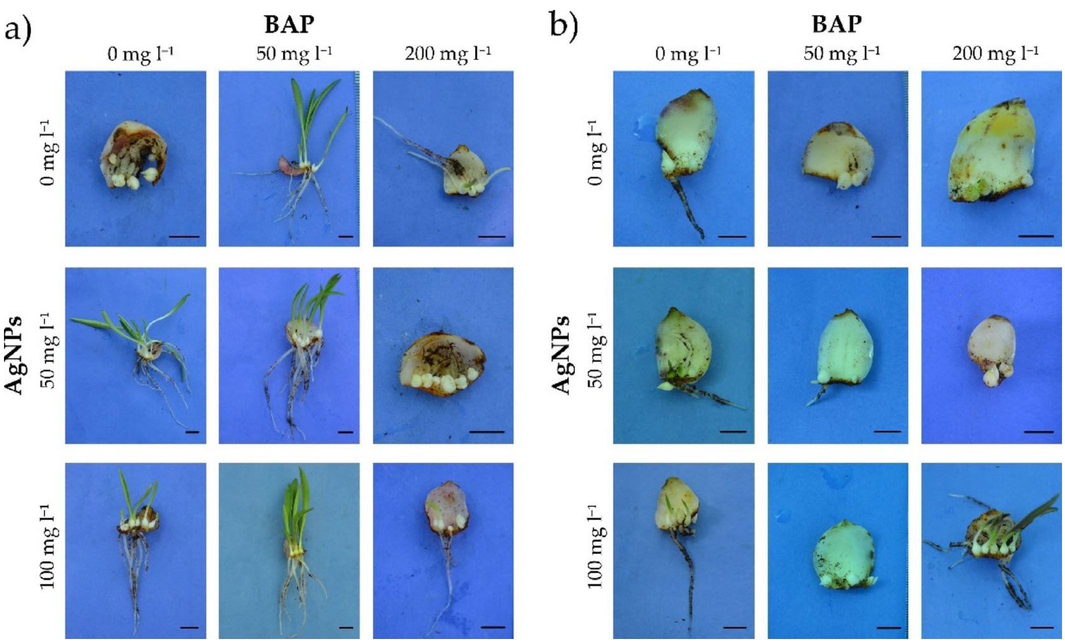

Fig. 2. Morphological comparisons of bulblet regeneration in the scales of two lily cultivars treated with different concentrations of AgNPs and BAP at day 45. (**a**) *Lilium* LA Hybrid 'Hyde Park' and (**b**) *Lilium* LA Hybrid 'Yellow diamond'. Black horizontal lines indicate scale = 1 cm.

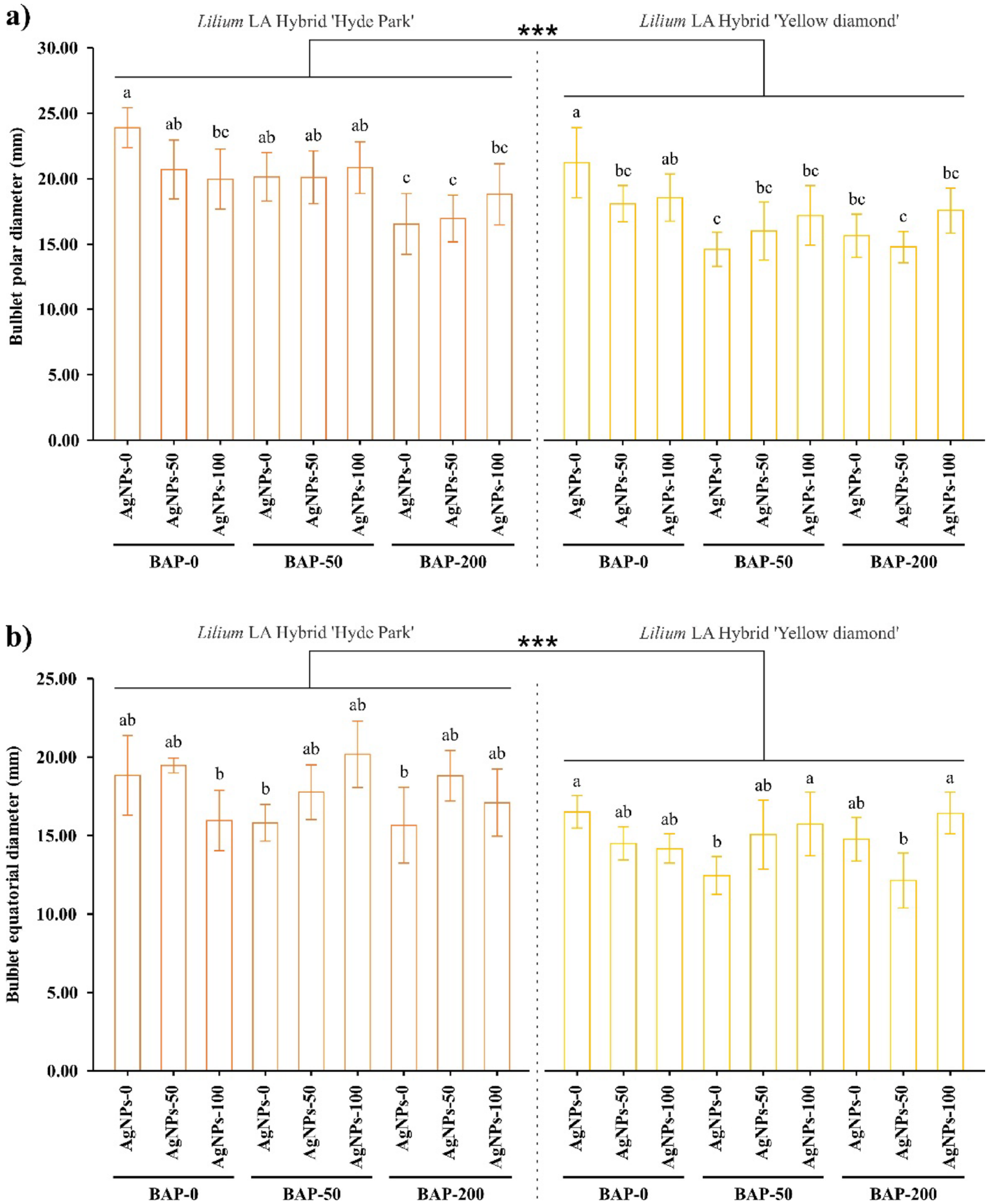

Fig. 3. Polar diameter (**a**) and equatorial diameter (**b**) of regenerated bulblet by two *Lilium* hybrids (LA Hybrid 'Hyde Park' and LA Hybrid 'Yellow diamond') treated with different concentrations of AgNPs and BAP. Data were analyzed with a Two-way ANOVA followed by Tukey's multiple comparison test. Values are presented as the mean ± Standard deviation. Different letters indicate significant differences at $p < 0.05$. Asterisks indicate significant differences between cultivars (*** $p < 0.001$).

underlying these observations, as the treatments could be modifying their morphology, which has direct implications on their quality and viability.

Bulblet weight

Two-way analysis of variance (ANOVA) showed a significant interaction effect between AgNPs and BAP on fresh weight of regenerated bulblets in both cultivars evaluated: LA Hybrid 'Hyde Park' ($F = 4.71$; $p < 0.001$) and LA Hybrid 'Yellow Diamond' ($F = 5.97$; $p < 0.001$). The highest bulblet weight in the two lily cultivars evaluated was obtained with the combination of AgNPs at 100 mg L^{-1} and BAP at 50 mg L^{-1}, registering 3.21 ± 0.75 g in 'Hyde Park' (Fig. 4, left side) and 2.42 ± 0.23 g in 'Yellow Diamond' (Fig. 4, right side). On the other hand, it was found that the combination of AgNPs with the highest concentration of BAP (200 mg L^{-1}) produced unfavorable effects on this parameter in both cultivars.

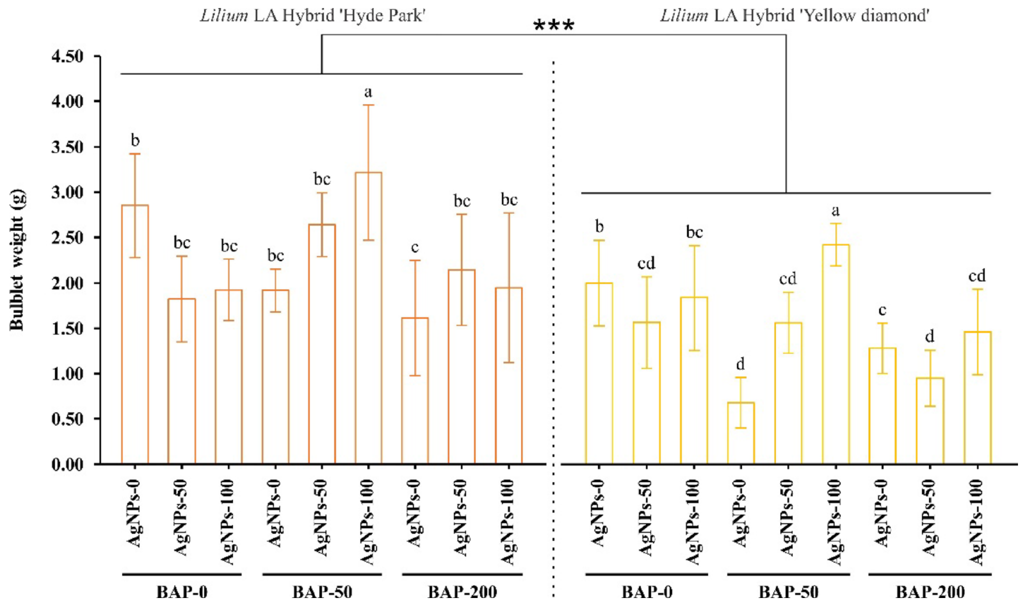

Fig. 4. Bulblet weight regenerated by two Lilium hybrids (LA Hybrid 'Hyde Park' and LA Hybrid 'Yellow diamond') treated with different concentrations of AgNPs and BAP. Data were analyzed with a Two-way ANOVA followed by Tukey's multiple comparison test. Values are presented as the mean ± Standard deviation. Different letters indicate a significant difference at $p < 0.05$. Asterisks indicate significant differences between cultivars (*** $p < 0.001$).

Plant height

Two-way analysis of variance (ANOVA) showed that the factors AgNPs and BAP had a significant effect on plant height in both cultivars evaluated: LA Hybrid 'Hyde Park' ($F = 8.91$; $p < 0.05$) and LA Hybrid 'Yellow Diamond' ($F = 6.85$; $p < 0.001$). In cv. 'Hyde Park', it was observed that application of BAP at 50 mg L^{-1} (145.46 ± 6.08 mm) maintained similar plant height to the control (141.31 ± 6.62 mm). This same comparable height pattern was found in plants treated with AgNPs at 100 mg L^{-1} in the absence of BAP (137.04 ± 10.54 mm) and in those receiving the combination of AgNPs at 100 mg L^{-1} and BAP at 50 mg L^{-1} (130.87 ± 14.54 mm) (Fig. 5, left side).

For cv. 'Yellow Diamond', application of AgNPs at 100 mg L^{-1} without BAP resulted in the greatest plant height (104.90 ± 15.29 mm). Notably, this height was not significantly different from that achieved when combining AgNPs (100 mg L^{-1}) with BAP (50 mg L^{-1}), which was 97.43 ± 7.24 mm (Fig. 5, right side). Overall, the results show that the response of lily cultivars to AgNPs and BAP is variable, suggesting a complex interaction between cultivar genetics and applied treatments.

Number of leaves and leaf area

The results of the two-way analysis of variance (ANOVA) indicated a significant effect of the factors AgNPs and BAP on leaf number of both cultivars: LA Hybrid 'Hyde Park' ($F = 8.98$; $p < 0.05$) and LA Hybrid 'Yellow Diamond' ($F = 2.64$; $p < 0.05$). In cv. 'Hyde Park' the treatment with BAP at 200 mg L^{-1} (without AgNPs) favored a higher leaf formation (average 5.5). However, the combination of AgNPs (50 and 100 mg L^{-1}) with BAP (50 and 200 mg L^{-1}) resulted in unfavorable effects on this parameter (Fig. 6a, left side). In cv. 'Yellow Diamond', plants treated with AgNPs at 50 mg L^{-1} in combination with BAP at 50 or 200 mg L^{-1} recorded similar leaf number with the control group (AgNPs-0 × BAP-0). In addition, high concentrations of AgNPs (100 mg L^{-1}) produced negative effects on leaf number (Fig. 6a, right side).

Regarding leaf area, the results indicate that in cv. 'Hyde Park' ($F = 22.48$; $p < 0.05$), the plants of the control group presented the highest mean leaf area (4699.2 ± 1248 mm^2), suggesting unfavorable effects of the combined application of AgNPs and BAP on this parameter (Fig. 6b, left side). For cv. 'Yellow Diamond' ($F = 24.23$; $p < 0.01$), the control treatments, AgNPs at 100 mg L^{-1} (without BAP) and the combination of AgNPs at 100 mg L^{-1} with BAP at 50 mg L^{-1} presented leaf area that did not differ significantly (between 2487.88 and 2765.9 mm^2) (Fig. 6b, right side).

Number and length of roots

Two-way analysis of variance (ANOVA) showed that the factors AgNPs and BAP had a significant effect on root number in both cultivars evaluated: LA Hybrid 'Hyde Park' ($F = 3.85$; $p < 0.001$) and LA Hybrid 'Yellow Diamond' ($F = 4.32$; $p < 0.01$). Figure 7a (left side) shows that, in cv. 'Hyde Park', treatment with AgNPs at 50 mg L^{-1} combined with BAP at 200 mg L^{-1} induced the highest number of roots, with a mean of 11.20 ± 1.64. It is observed that, when using BAP at a concentration of 50 mg L^{-1}, the increase in AgNPs doses seems to exert a potentially beneficial effect on root formation. However, the combination of high concentrations of both BAP and AgNPs shows an inhibitory effect on root numbers. As for cv. 'Yellow Diamond' (Fig. 7a, right side), the highest number of roots was recorded in plants treated with AgNPs at 100 mg L^{-1} together with BAP at 50 mg

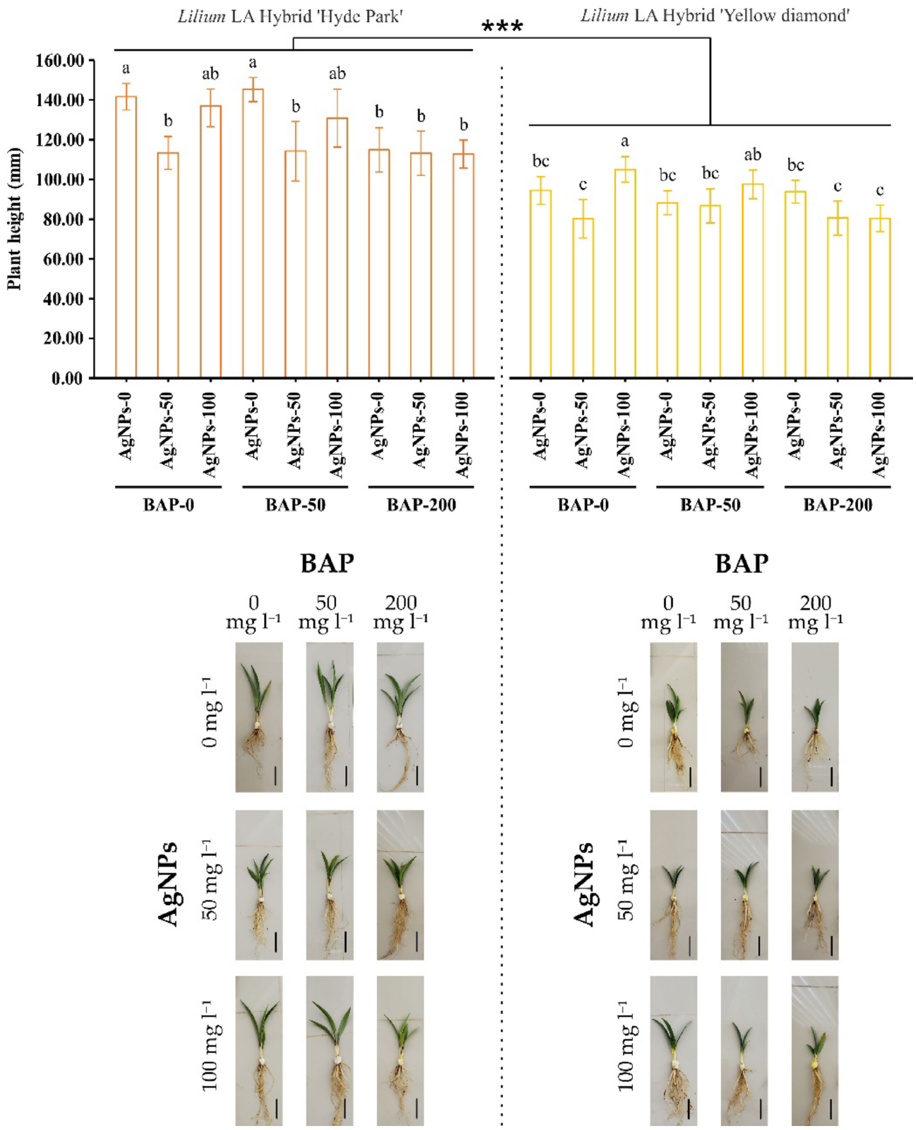

Fig. 5. Plant height recorded by two *Lilium* hybrids (LA Hybrid 'Hyde Park' and LA Hybrid 'Yellow diamond') treated with different concentrations of AgNPs and BAP. Data were analyzed with a Two-way ANOVA followed by Tukey's multiple comparison test. Values are presented as the mean ± Standard deviation. Different letters indicate a significant difference at $p < 0.05$. Asterisks indicate significant differences between cultivars (*** $p < 0.001$). Black vertical lines indicate scale = 5 cm.

L^{-1} (9.4 ± 1.52). Like what was found in 'Hyde Park', the increase in AgNPs concentrations suggests a potentially favorable effect on root development.

Root length analysis in cv. 'Hyde Park' ($F = 2.57$; $p < 0.05$) shows that treatments with AgNPs at 50 mg L^{-1} together with BAP at 200 mg L^{-1} and AgNPs at 100 mg L^{-1} combined with BAP at 50 mg L^{-1}, promoted the greatest root lengths, reaching a mean of 224.08 mm and 222.93 mm, respectively (Fig. 7, left side). On the other hand, for cv. 'Yellow Diamond' ($F = 8.60$; $p < 0.001$), the treatment with AgNPs at 100 mg L^{-1}, without the addition of BAP, resulted in greater root length development (202.464 ± 59.9 mm). In this cultivar, the combination of AgNPs with BAP showed unfavorable results for this parameter (Fig. 7b, right side).

Physiological indicators

Relative chlorophyll content (SPAD)

The results of the two-way analysis of variance (ANOVA) indicated a significant effect of the factors AgNPs and BAP on the SPAD index of leaves of cv. LA Hybrid 'Hyde Park' ($F = 2.37$; $p < 0.05$), in contrast to cv. LA Hybrid 'Yellow Diamond' ($F = 2.03$; $p > 0.05$). In cv. 'Hyde Park', the control treatment presented the highest SPAD index value (62.28 ± 2.68) (Fig. 8, left side). On the other hand, in cv. 'Yellow Diamond', SPAD values ranged from 63.3 to 75.24 (Fig. 8, right side) without showing significant differences between treatments.

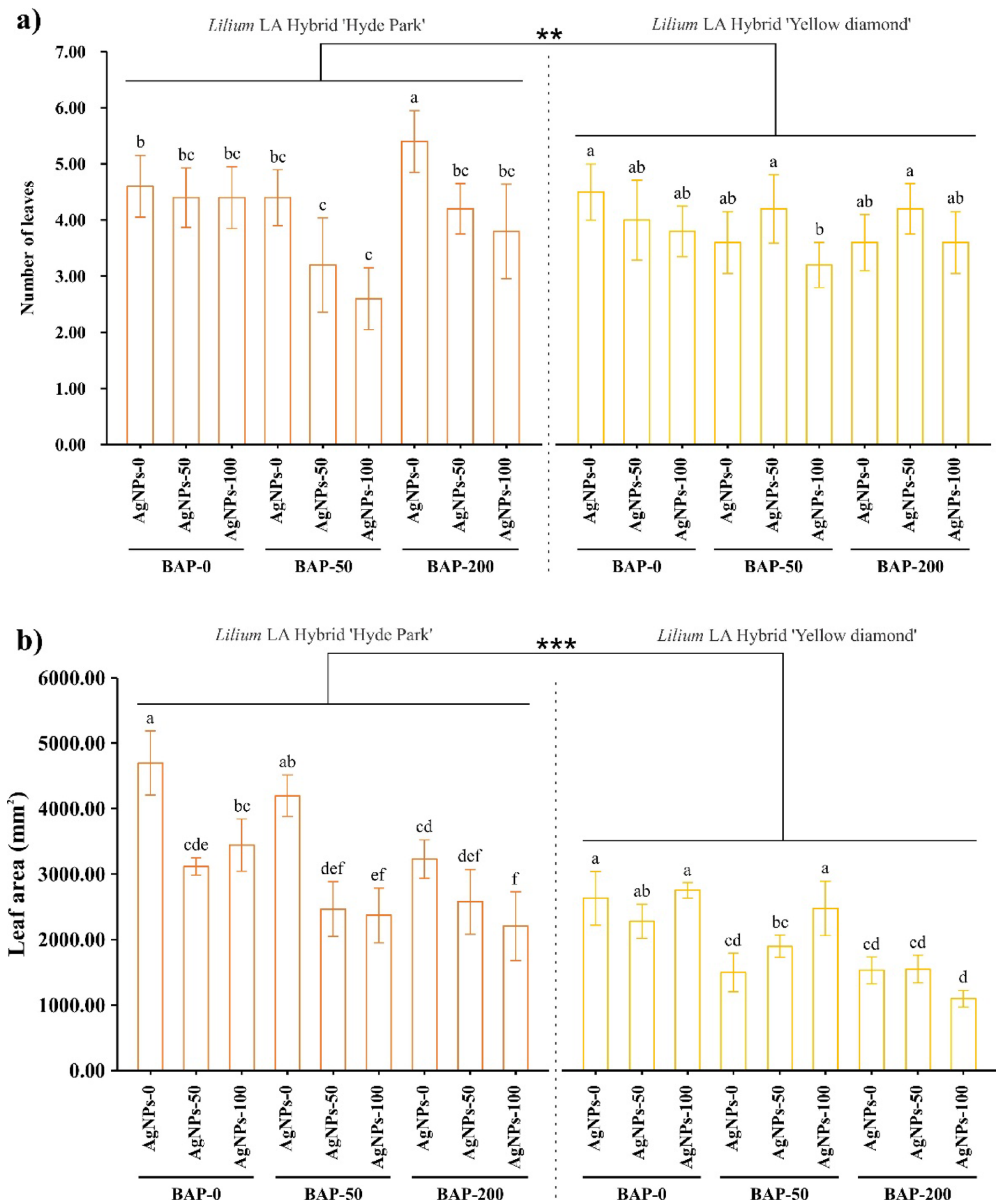

Fig. 6. Number of leaves (**a**) and leaf area (**b**) recorded by two *Lilium* hybrids (LA Hybrid 'Hyde Park' and LA Hybrid 'Yellow diamond') treated with different concentrations of AgNPs and BAP. Data were analyzed with a Two-way ANOVA followed by Tukey's multiple comparison test. Values are presented as the mean ± Standard deviation. Different letters indicate a significant difference at $p < 0.05$. Asterisks indicate significant differences between cultivars (** $p < 0.01$; *** $p < 0.001$).

Stomatal conductance

Two-way analysis of variance (ANOVA) showed that the factors AgNPs and BAP had a significant effect on stomatal conductance in both cultivars evaluated: LA Hybrid 'Hyde Park' ($F = 7.78$; $p < 0.001$) and LA Hybrid 'Yellow Diamond' ($F = 7.80$; $p < 0.001$). In cv. 'Hyde Park', the highest value of stomatal conductance (545.62 ± 106.16 mmol/m^2 s) was recorded in plants treated with the combination of AgNPs at 50 mg L^{-1} with BAP at 200 mg L^{-1}. In contrast, the control treatment (AgNPs-0 × BAP-0) and the application of AgNPs at 100 mg L^{-1} without BAP showed the lowest values of stomatal conductance, with 272.84 ± 68.90 mmol/m^2 s and 263.5 ± 66.81 mmol/m^2 s, respectively (Fig. 9, left side).

In cv. 'Yellow Diamond', the highest stomatal conductance (600.96 ± 132.52 mmol/m^2 s) was recorded in the treatment with BAP at 50 mg L^{-1} without AgNPs, being significantly higher compared to the other treatments (Fig. 9, right side).

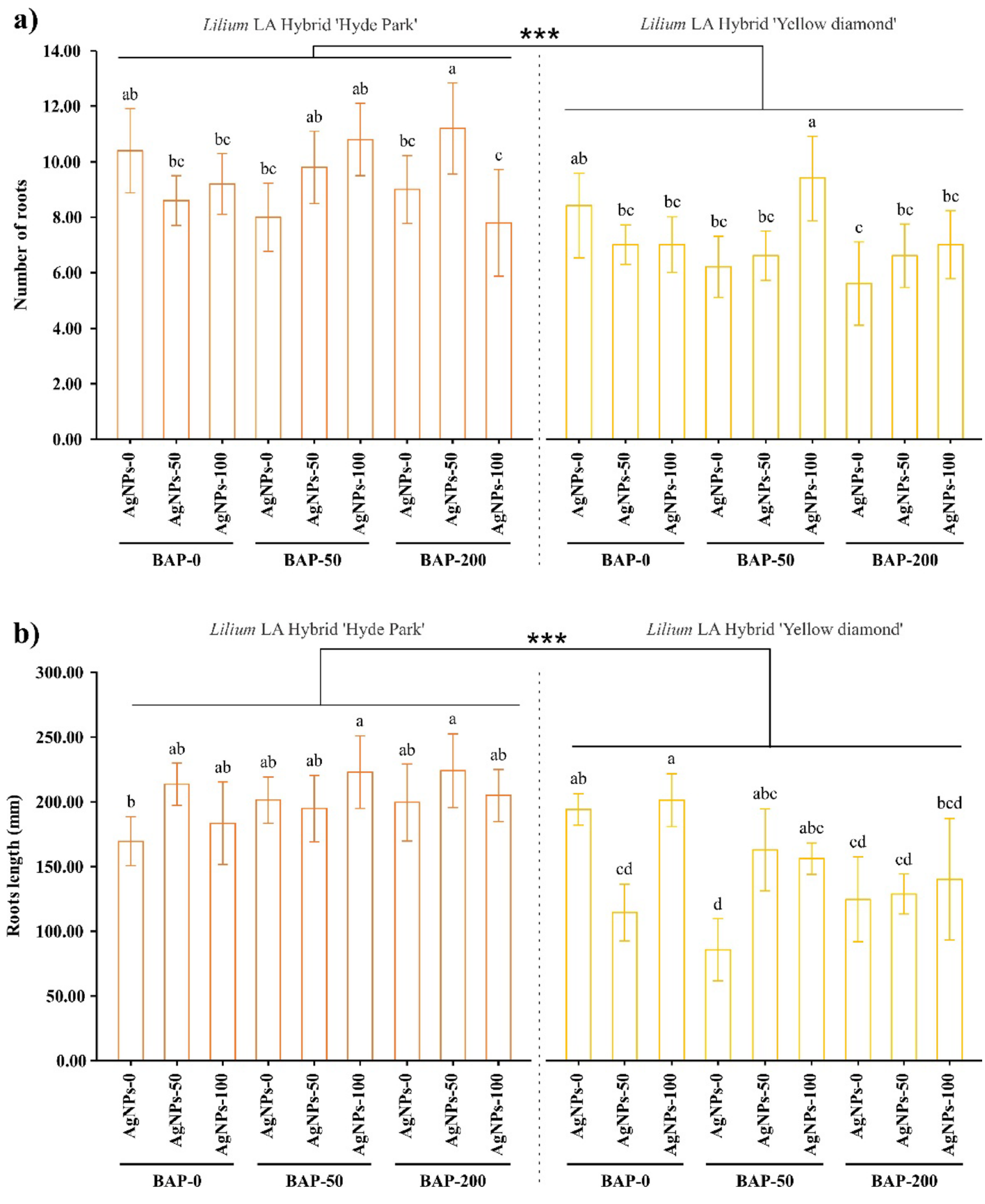

Fig. 7. Number (**a**) and length of roots (**b**) recorded by two *Lilium* hybrids (LA Hybrid 'Hyde Park' and LA Hybrid 'Yellow diamond') treated with different concentrations of AgNPs and BAP. Data were analyzed with a Two-way ANOVA followed by Tukey's multiple comparison test. Values are presented as the mean ± Standard deviation. Different letters indicate a significant difference at $p < 0.05$. Asterisks indicate significant differences between cultivars (*** $p < 0.001$).

Correlation analysis

Heat maps provide a visualization of the correlations between the parameters examined for each lily cultivar (Fig. 10). In cv. 'Hyde Park' (Fig. 10a), leaf area showed a moderate positive correlation with SPAD index (r = 0.57), while it had a negative correlation with stomatal conductance (r = -0.5). Plant height was moderately positively correlated with bulblet polar diameter (r = 0.59). In cv. 'Yellow Diamond' (Fig. 10b), bulblet polar diameter was strongly positively correlated with bulblet weight (r = 0.63), followed by number of roots (r = 0.62). The SPAD index was moderately positively correlated with the number of roots (r = 0.54).

Discussion

It has been reported that bulb scales, have an excellent capacity to regenerate new bulblets, due to their high nutrient and water content[22]. In this context, optimizing the propagation and cultivation processes of lily is possible thanks to the tools provided by state-of-the-art technologies[23], such as in vitro culture, genetic engineering or nanotechnology.

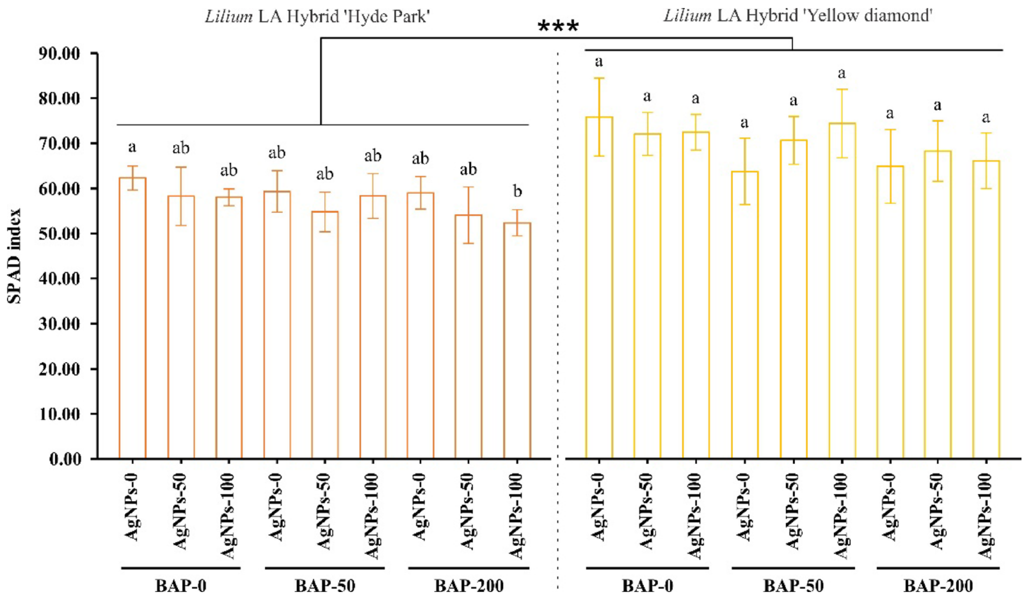

Fig. 8. Relative chlorophyll content (SPAD) recorded by two *Lilium* hybrids (LA Hybrid 'Hyde Park' and LA Hybrid 'Yellow diamond') treated with different concentrations of AgNPs and BAP. Data were analyzed with a Two-way ANOVA followed by Tukey's multiple comparison test. Values are presented as the mean ± Standard deviation. Different letters indicate a significant difference at $p < 0.05$. Asterisks indicate significant differences between cultivars (*** $p < 0.001$).

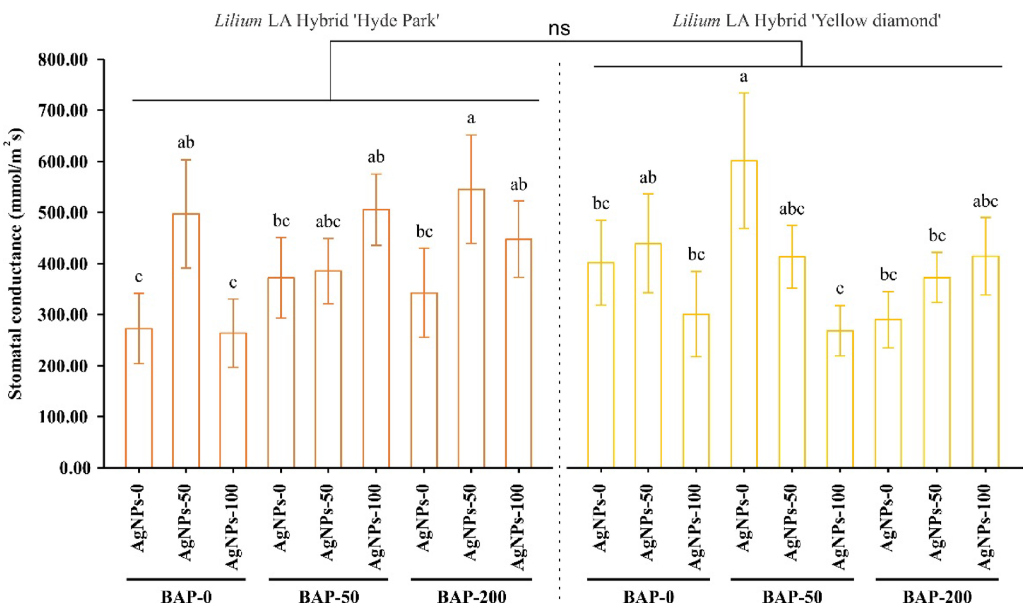

Fig. 9. Stomatal conductance recorded by two *Lilium* hybrids (LA Hybrid 'Hyde Park' and LA Hybrid 'Yellow diamond') treated with different concentrations of AgNPs and BAP. (**a**) *Lilium* LA Hybrid 'Hyde Park' and (**b**) *Lilium* LA Hybrid 'Yellow Diamond'. Data were analyzed with a Two-way ANOVA followed by Tukey's multiple comparison test. Values are presented as the mean ± Standard deviation. Different letters indicate a significant difference at $p < 0.05$. ns, no significant differences at $p < 0.05$.

Morphological indicators

The development of new bulbs is directly related to the endogenous content of hormones and carbohydrates, being factors that influence the number of regenerated bulbs, number of leaves and plant height[24]. In line with this, Lapiz-Culqui et al.[5], pointed out that the process of proliferation and development of new bulblets in bulbous plants is influenced by the exogenous application of growth regulators. The formation of new bulbs, is similar to the growth of axillary buds, since they initially manifest themselves in this way and gradually differentiate until they form a new bulb, i.e., it is a process that is controlled by different hormones that induce

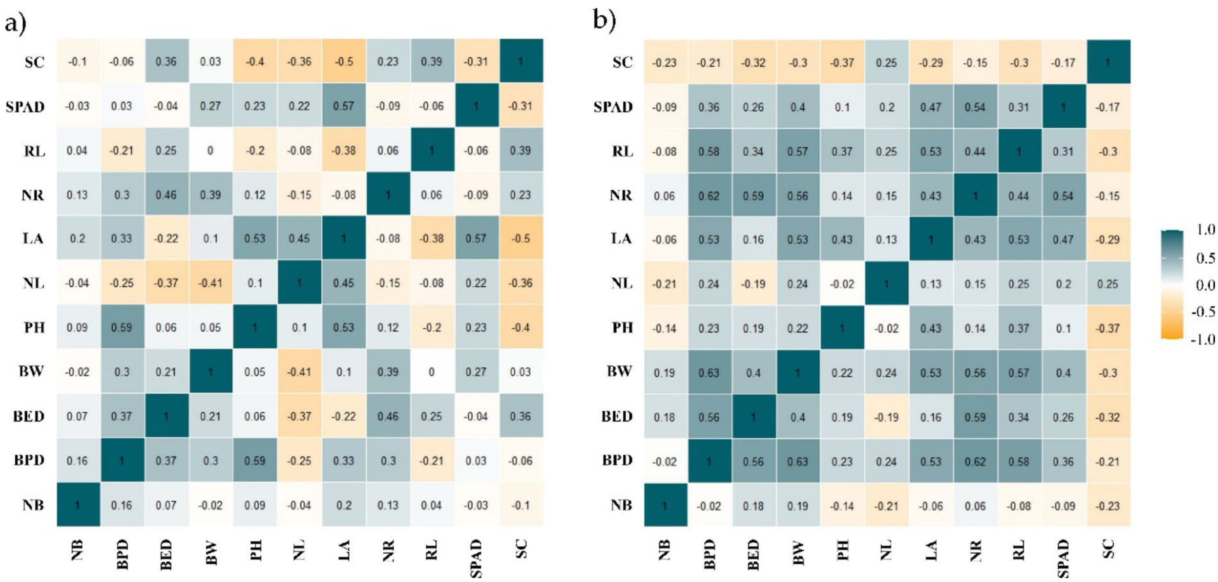

Fig. 10. Hierarchical heat map of Pearson's correlation coefficients between the different parameters evaluated for two lily cultivars: (**a**) *Lilium* LA Hybrid 'Hyde Park' and (**b**) *Lilium* LA Hybrid 'Yellow Diamond'. The dendrograms represent the hierarchical grouping of variables based on their correlations. Variables evaluated include NB: number of bulblets, BPD: bulblet polar diameter, BED: bulblet equatorial diameter, BW: bulblet weight, PH: plant height, NL: number of leaves, LA: leaf area, NR: number of roots, RL: root length, SPAD: chlorophyll index, SC: stomatal conductance.

cell division[25,26]. The present investigation revealed a highly variable response in cv. 'Hyde Park' and cv. 'Yellow Diamond' to the application of BAP and AgNPs. In certain treatments, a significant improvement in bulblet induction and an acceleration of bulblet development was observed, as evidenced by leaf formation at 45 days. However, the application of these compounds did not always result in an increase in the formation of new propagules, highlighting the considerable variability characterizing biological experiments.

Previous studies, such as those reported by Mo et al.[27], revealed that exogenous application of 50 mg L^{-1} of BAP increases the rate of bulblet formation in *L. lancifolium*. Nevertheless, AgNPs have exhibited both favorable and adverse effects, generating cytotoxicity and genotoxicity in new propagules[28,29]. The impact depends on the concentration, size and shape of the nanoparticles[30,31]. Consequently, numerous studies have consistently revealed that smaller nanoparticles (< 50 nm) are more detrimental to cultures[32,33], because they block plasmodesmata through their transport via symplastic pathway, an effect that is exacerbated by their irregular shapes[34,35].

The results obtained suggest that treatment with AgNPs in bulb flakes had a significant effect on the formation of new bulblets, weight and morphological development of new propagules of lily cultivars 'Hyde Park' and 'Yellow Diamond'. Byczyńska et al.[36] reported beneficial properties and observed that soaking *Lilium* 'Bright Pixi' scales in 100 mg L^{-1} of AgNPs increased bulb yield, plant height and fresh weight. In addition, it has been indicated that AgNPs, act synergistically with some growth regulators to increase plant vigor and propagative capacity under certain adverse effects of biotic and abiotic stresses[37]. Likewise, previous studies found that soaking tulip bulbs in AgNPs also promoted faster flowering, longer stems, larger petals, and greater stem diameter[38].

Previous studies have also reported that treatment of bulb scales with 100 mg L^{-1} of AgNPs stimulates the formation of new bulblets with improved adventitious root development[39]. In addition, bulbs treated with AgNPs are more resistant to infections caused by soil pathogens[8]. As a result, plants grown from these bulbs, have a more vigorous development and growth from the beginning of their cultivation[2].

However, while AgNPs treatment offers benefits, it is necessary to recognize that it can also induce adverse effects on root development. For example, in *Arabidopsis thaliana*, a significant reduction in the number and length of radicular hairs has been observed in plants treated with AgNPs[29]. This effect is due to the fact that Ag^{+} ions released from AgNPs are higher in the rhizosphere[17]. According to Khan et al.[40] these adverse effects are mainly caused by applications in excessive concentrations. As previously demonstrated in *Ananas comosus* L., a high accumulation of Ag in plant tissues reduces the morphological characteristics of the plants[16].

It should also be remembered that, in addition to hormones, the formation of new bulblets in lily scales is closely related to the nutritional quality of the mother bulb[26]. Previous studies have shown that degradation of starch stored in the scales provides the soluble sugars, mainly sucrose, necessary for bulblet growth and development[41–43]. Therefore, the selection of healthy bulbs with high levels of starch reserves also plays an essential role in ensuring efficient vegetative propagation and obtaining high quality bulblets.

This research highlights the importance of determining optimal concentration for the synergistic use of AgNPs and BAP. Adverse outcomes may occur if concentration is not adjusted, as the response varies by species, variety, cultivar, or genotype.

Physiological indicators

Recent findings indicate that NPs possess the ability to modulate plant growth and development through their interaction with hormonal signaling networks, generating regulatory responses that can be both complementary and antagonistic[21]. This phenomenon, known as signal crosstalk, ultimately leads to the reprogramming of gene expression and plant physiology[44]. In this context, AgNPs have been specifically reported to modify the hormonal levels of the main groups of phytohormones (cytokinins, auxins and gibberellins)[45].

In this study, the application of growth regulators (BAP) and nanoparticles (AgNPs) to the scales of lily cultivars during sowing did not produce relevant changes in SPAD values with respect to the control treatments. However, it has been recorded that these products are absorbed and assimilated by the scales, which can triggers metabolic, biochemical and physiological processes that favor leaf development[46,47]. At the physiological level, they activate the primary metabolism of seedlings such as photosynthesis, nutrient absorption, among others[48]. It should be noted that SPAD values are related to the increase in leaf area and the concentration of photosynthetic pigments and carotenoids[49].

Several investigations have reported that the application of 100 mg L^{-1} of AgNPs improves the photosynthetic activity of lilies and tulips, favoring the accumulation of higher chlorophyll concentrations[2,38], and thus SPAD indices. Similar effects, were reported in cultivars of *Phaseolus vulgaris*[50], *Brassica juncea*[51], *Solanum tuberosum*[52], *Solanum lycopersicum*[53] y *Lilium* cv. 'Mona Lisa' y cv. 'Little John'[2]. On the other hand, growth regulators such as BAP have also been described to enhance chlorophyll content[54].

Despite this, while AgNPs offer promising applications, it cannot be ruled out that their excessive use may negatively affect cell structure, photosynthetic activity and transpiration rate[55,56]. Nanoparticles can cause oxidative damage by mainly increasing the production of reactive oxygen species (ROS), affecting the functionality of chloroplasts and triggering a series of physiological disturbances[57,58]. It should also be noted that the toxicity of nanoparticles depends on the method used for their production[59,60], with biosynthesis from plant tissues emerging as an innovative alternative to minimize the toxic effects of AgNPs on crops in general[61].

The analysis of stomatal conductance in lily cultivars revealed that the joint application of AgNPs and BAP, under specific concentration conditions, promoted an increase in the values of this parameter. Similar results were described by Byczyńska et al.[38], evidencing that the application of 100 mg L^{-1} of AgNPs in tulips, increases the values of stomatal conductance during seedling development. Likewise, silicon NPs have been reported to increase stomatal conductance in *Coriandrum sativum* L. plants under salt stress[62]. In part, the increase or decrease in stomatal conductance values indicate that plants are coping with stressful situations to adapt to different natural conditions[63].

The interaction between NPs and cytokinins, evidenced in a study performed in Arabidopsis thaliana, allowed a significant increase in the transcription levels of *ARR7* and *ARR15*, key genes in the cytokinin signaling pathway, which resulted in an increase in the content of photosynthetic pigments in plants[64]. This finding supports the existence of distinct but complementary mechanisms of action between the two modulators[21]. While cytokinins such as BAP act directly as a signaling hormone that activates genetic pathways associated with cell division and metabolism[65]; NPs intervene in several processes simultaneously: they optimize nutrient uptake and confer protection against stress-induced oxidative damage[66], functions that cytokinins alone cannot provide. Thus, it is argued that, although the exclusive application of BAP favors the proliferation of new propagules, it does not guarantee, for example, adequate root development. In the present study, the combination of both factors resulted in more vigorous roots, leading to the generation of new bulbs that gave rise to more robust and viable seedlings.

Conclusions

The study showed that the cultivar 'Hyde Park' has a higher bulblet regeneration capacity compared to the cultivar 'Yellow Diamond', producing at least 5 bulblets per scale in the former case. The observed response to AgNPs and BAP treatments reveals a complex interaction between these compounds, whose effect is highly dependent on the concentration used and the lily cultivar. Specifically, in cv. 'Hyde Park', the combination of AgNPs at 50 mg L^{-1} with BAP at 200 mg L^{-1} maximized bulblet formation. In contrast, in cv. 'Yellow Diamond', the treatments that showed a significant influence on regeneration were the combination of AgNPs at 100 mg L^{-1} with BAP at 200 mg L^{-1}, as well as the treatment with AgNPs at 50 mg L^{-1} in the absence of BAP. In terms of height, 'Hyde Park' reached its maximum development with the application of BAP at 50 mg L^{-1} without AgNPs. On the other hand, 'Yellow Diamond' showed its greatest height with AgNPs treatment at 100 mg L^{-1} in the absence of BAP. In general, the results obtained show a highly varietal response to the application of AgNPs and BAP. This underscores that there is no single optimal combination universally effective for all parameters evaluated nor for all cultivars. This specificity implies that effects can be both beneficial and adverse, depending critically on the treatment conditions applied. Therefore, it is crucial to meticulously adjust the doses and combinations of AgNPs and BAP in order to optimize regeneration and growth in lily production, considering the intrinsic particularities of each cultivar and the desired agronomic objective (e.g., number or weight of bulblets, or plant height). Additionally, it is recommended to evaluate the phenotype of plants treated with AgNPs in subsequent studies, given the possibility that these nanoparticles may exert genotoxic and mutagenic effects.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

Conceptualization, OTL and JBMM; methodology, JJTA and YKLC; formal analysis, EHH and MZP; investigation, OTL and RSMP; data curation, MOC and MZP; writing—original draft preparation, JBMM and RSMP; writing—review and editing, JJTA, YKLC and EHH; supervision, MOC. All authors have read and agreed to the published version of the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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