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Effect of gibberellin on *in vitro* bulblet induction of lily (*Lilium orientalis* L. cv. Santander)

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ABSTRACT

Purpose: Lily is one of the most economically ornamental plants and tissue culture plays a vital role in accelerating mass propagation of lily. In lily tissue culture, the production of bigger bulblets is highly desirable. The objective of the present investigation is to examine the impact of gibberellin on the *in vitro* growth of lily bulblets, administered at two distinct time intervals. **Research method:** In the present investigation, various concentrations of gibberellins (0, 0.1, and 1 µM) were employed at two distinct time points: the commencement of the culture period and the fifth week of culture period. After 11 weeks the fresh weight of bulblets, the number of bulblets, the fresh weight of leaves, the fresh weight of roots and the fresh weight of scale explant were scored and analyzed. **Findings:** The application of 1 µM gibberellin during bulblet induction yielded noteworthy outcomes, including a substantial 91% increase in the fresh weight of the bulblets, a significant 38% augmentation in the fresh weight of the leaves, as well as a 40% increase in the fresh weight of the roots. **Research limitations:** The quantification of endogenous phytohormones in lily scale explants was deemed unfeasible**. Originality/value:** The development of lily bulblets experienced a notable enhancement while the medium was supplemented with gibberellin in bulblet induction stage.

INTRODUCTION

Lilium is a genus of herbaceous flowering plants that originate from bulbs and belong to the lily family (*Liliaceae*) (Askari et al., 2018). The lily has gained significant popularity as a cut flower worldwide, accounting for approximately 25% of the total export value of flower bulbs (Ahmed et al., 2018). The breeding process for lilies is a time-consuming endeavor, with the introduction of a newly bred lily cultivar to the market historically taking up to 15 years. The initial phase of this process involves the selection of the most desirable clone, which requires several years. From the time of seed germination to the development of a flowering plant, approximately three years' elapse. Consequently, the evaluation of flower properties can only occur several years after the initial crosses have been made. Subsequently, additional years are necessary to assess quantitative traits such as yield and disease resistance. Once the best clone has been identified, the production of an adequate number of bulbs requires several more years due to the slow pace of available vegetative propagation methods, such as natural propagation and (artificial) "scaling" (Grassotti & Gimelli, 2011). However, the application of micropropagation has significantly reduced this propagation period and is now widely employed in most breeding programs (Askari et al., 2018). Through *in vitro* techniques, a large number of genetically identical bulblets can be produced from a single bulb within a short timeframe. As a result of the high propagation rates achieved through tissue culture, newly bred cultivars can be introduced to the market within a few years (typically 7-8 years) (Benschop et al., 2010). Consequently, tissue culture plays a crucial role in the rapid expansion of the lily assortment observed in contemporary times (Langens-Gerrits et al., 1997; Langens-Gerrits et al., 2003). The production of bulblets and other storage organs in *in vitro* condition has been observed to exhibit favorable characteristics as propagules. These propagules possess the advantages of being easily controllable, transportable, and storable, thereby eliminating the need for elaborate acclimatization procedures upon their transfer to soil (Askari et al., 2016; Thakur et al., 2006). In the micropropagation process of lilies, bulblets are generated as the final stage (Youssef et al., 2019). The size of the bulblets produced *in vitro* has a significant impact on their performance after planting. Studies have shown that small bulblets exhibit slower emergence, less uniformity, and a lower percentage of sprouting (Askari & Visser, 2022). Therefore, in tissue culture, it is recommended to produce heavy bulblets to ensure better performance after planting (Askari & De Klerk, 2020). Gibberellin is a category of plant hormones that promotes stem elongation, flowering, and germination (Li et al., 2020). These hormones are produced through the terpenoid pathway in plastids and undergo modifications in the endoplasmic reticulum and cytosol to attain their biologically active state (Castro-Camba et al., 2022). In tissue culture, gibberellins are employed to stimulate organogenesis, specifically the formation of adventitious roots (Ahmad et al., 2020; Willy John, 2022). Gibberellins have been discovered to hinder the process of tuberization in potatoes, instead inducing stolon to elongate rather than enlarge. Moreover, they impede the accumulation of starch and the synthesis of proteins that are specific to tubers in potatoes (Vreugdenhil & Sergeeva, 1999). The examination of the influence of inhibitors of gibberellin synthesis (Alar, Cycocel and Paclobutrazol) on the growth and development of oriental lily hybrids has been carried out, and it has been observed that the utilization of these growth retardants leads to enhance bulblet formation (Kumar et al., 2005). On the other hand, the *ex vitro* scaling of *Lilium davidii var. unicolor* was observed to have a beneficial impact due to the application of gibberellin. Scales treated with a concentration of 100 mg/L GA_3 resulted in a greater number of bulblets compared to the control. Additionally, the diameter of the bulblets was increased by GA_3 at both 100 and 150 mg/L (Tang et al., 2020). Additionally according to the findings of Ren et al. (2021), the

levels of endogenous gibberellic acid (GA_3) exhibited a notable rise during the competence stage (0–1 day) of culturing scale explants of Lycoris sprengeri in tissue culture. Subsequently, as the bulblet development progressed, the concentration of GA_3 gradually declined. Furthermore, a research revealed that during the *in vitro* rooting procedures of *Carpinus betulus*, the concentration of endogenous GA_3 reached its highest point in the middle of the rooting phase, whereas the IAA/ABA ratio declined in the middle of the adventitious rooting period (Zhu et al., 2017). In Bougainvillea, also the concentration of endogenous GA increased during the adventitious root induction (Huang et al., 2022). Due to the increasing and decreasing of endogenous gibberellin in different stages of adventitious organ formation (bulblet and adventitious root), as well as the lack of information about the impact of gibberellin on lily bulblet initiation and induction individually, this study was conducted to investigate the effects of gibberellin at two distinct time intervals (a) at the commencement of the experiment (bulblet initiation) and b) after the fifth week of the experiment (bulblet induction). This evaluation aims to determine whether gibberellin negatively affects bulblet initiation or it hampers bulblet induction in lily bulblet growth *in vitro.*

MATERIALS AND METHODS

Standard tissue culture conditions

The present study utilized field-grown bulbs of *Lilium orientalis* L. cv. Santander with a circumference of 18-20 cm. These bulbs were harvested, subjected to cold treatment to break dormancy, and stored at -1.0 °C until further use, as previously described by Askari et al. (2014). Prior to use, scales were surface-sterilized for 30 minutes in 1% (w/v) NaClO and rinsed for 1, 3, and 10 minutes with sterile water, as per the protocol established by Askari and De Klerk (2018).The sterilized scales were then stored in sterile water for an average of 1-2 hours until use. Explants were cut into standard (7×7 mm^2) sizes. The abaxial side of the explants (one explant per container) was placed on the medium in the container (red cap plastic container (2.5×5 cm), which was composed of macro- and microelements (Murashige & Skoog, 1962), 30 g l^{-1} sucrose, 0.4 mg l^{-1} thiamine, 100 mg l^{-1} myo-inositol, 7 g l^{-1} microagar (Fig. 1). The explants were maintained under standard growth conditions, which involved adjusting a temperature of 25°C and a light intensity of 30 µmol m^{-2} sec^{-1} (Philips TL 33) for 16 hours per day (Askari et al., 2022).

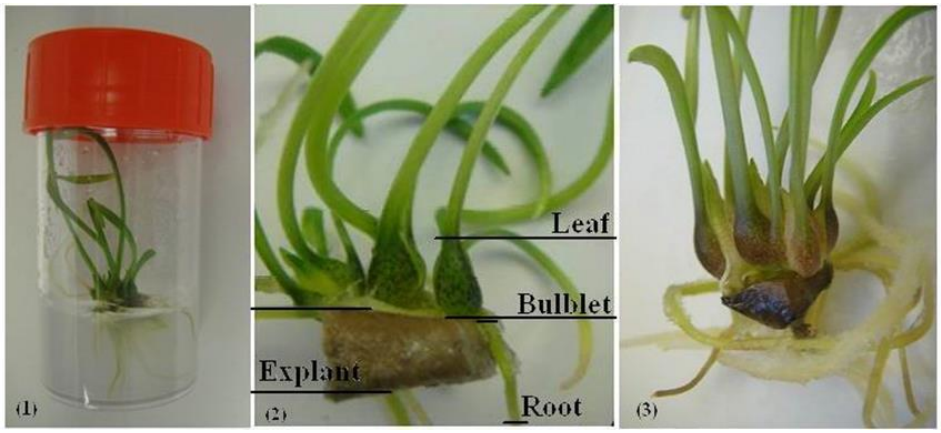

Fig. 1. The in vitro growth of Lily bulblets was observed through the following procedures. (1) Tissue culture containers including 15 ML MS medium, (2) Various Lily organs regenerated on the scale explant, and (3) The growth of Lily bulblets for duration of 11 weeks.

Gibberellins treatments

The experimental design encompassed two factors: 1) varying concentrations of gibberellins, specifically 0, 0.1, and 1 µM gibberellin (GA_3), and 2) two distinct application times, including a) at the commencement of the experiment (bulblet initiation) and b) after the fifth week of the experiment (bulblet induction). The experiment involved three replications and ten replicates for each treatment. To assess the impact of gibberellin on lily bulblet induction, a total of 60 explants were cultured on free hormone Murashige and Skoog medium (MS) (Murashige & Skoog, 1962): containing 30 g L^{-1} sucrose, 0.4 mg L^{-1} thiamine; 100 mg L^{-1} myo-inositol; 7 g L^{-1} microagar; and maintained at a temperature of 25±2 ºC and 30 µmol m^{-2} s^{-1} light (Philips TL 33) for 16 h per day for five weeks. After 5 weeks, the scale explants were subcultured on the same MS medium containing 0.1 and 1 µM gibberellin and incubated at the same condition for further growth. For evaluation of the effect of gibberellin on initiation of lily bulblets *in vitro* the scale explants were cultured on the MS medium (above mentioned) containing 0, 0.1, and 1 µM gibberellin from the beginning of the experiment. In order to eliminate the influence of subculturing on the results of the experiments, the explants that were subjected to gibberellin (0, 0.1, and 1 µM) at the beginning of the experiments were also subcultured simultaneously (at week five) onto the same medium.

Data collection

Following an 11-week growth period, measurements were taken of the fresh weights of bulblets, the number of bulblets per explant, the fresh weight of leaves and roots per explant, as well as the fresh weight of the explant itself. All the fresh weigh measured with analytical balance.

Statictis

The present investigation was executed as a factorial design in accordance with the principles of a completely randomized design (CRD), involving two factors: factors: 1) varying concentrations of gibberellins, specifically 0, 0.1, and 1 µM gibberellin (GA_3), and 2) two distinct application times, including a) at the commencement of the experiment (bulblet initiation) and b) after the fifth week of the experiment (bulblet induction). Data analysis was performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). Specifically, a one-way ANOVA was employed to identify significant differences between individual treatments, with the Duncan test utilized to determine such differences. It should be noted that values sharing the same letter were found to not differ significantly at the 0.05 level. In the figures, the means are shown ± SE.

RESULTS

The impact of gibberellin on the growth of lily bulblets

The findings of this study indicate that the fresh weight of lily bulblets is influenced by varying concentrations of gibberellin, as depicted in Figure 2. Specifically, the addition of gibberellin to the culture medium at the initiation stage resulted in a 26% increase in the fresh weight of the bulblets. In addition, the introduction of gibberellin into the growth medium after the fifth week of the experiment (induction stage) resulted in a significant 91% increase in the fresh weight of the bulblets, particularly when exposed to a concentration of 1 µM gibberellin (Fig. 2). These results show that the timing of gibberellin application significantly impacts bulblet growth *in vitro.* Notably, adding gibberellin at the induction stage led to a 51% improvement in bulblet growth compared to adding gibberellin at the onset of the experiment (initiation stage). The application of gibberellin at the onset of the experiments did

not yield a remarkable impact on the growth of bulblets in 0.1 and 1 µM gibberellin concentration. However, the application of gibberellin after the fifth week alters the growth of lily bulblets, depending on the concentration used. Specifically, a higher concentration of gibberellin (1 µM) led to the production of larger bulblets (94 mg/bulblet) compared to the lower concentration of gibberellin (0.1 µM).

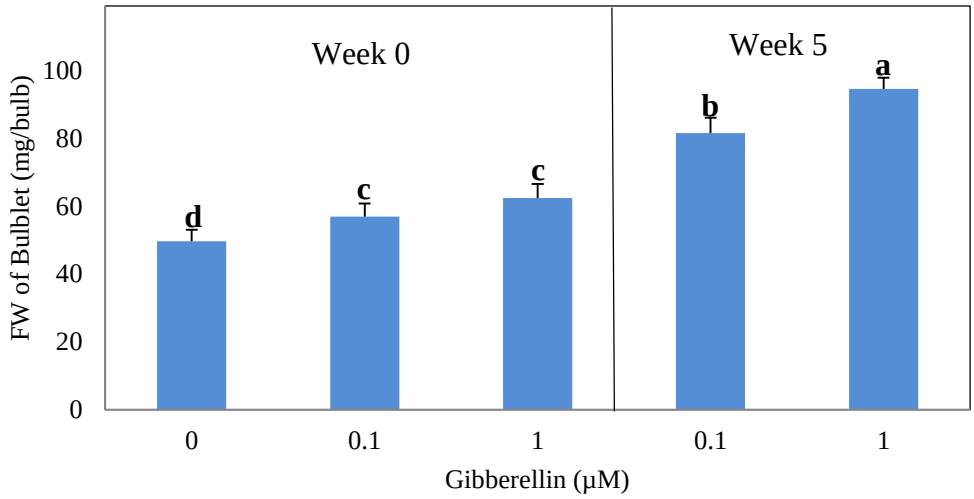

Fig. 2. Effect of different concentrations of gibberellin on FW (fresh weight) of bulblets. The error bar represents the standard error. The presence of letters above the error bars, as determined by Duncan's multiple range test, indicated the differences observed among the treatments (p=0.05).

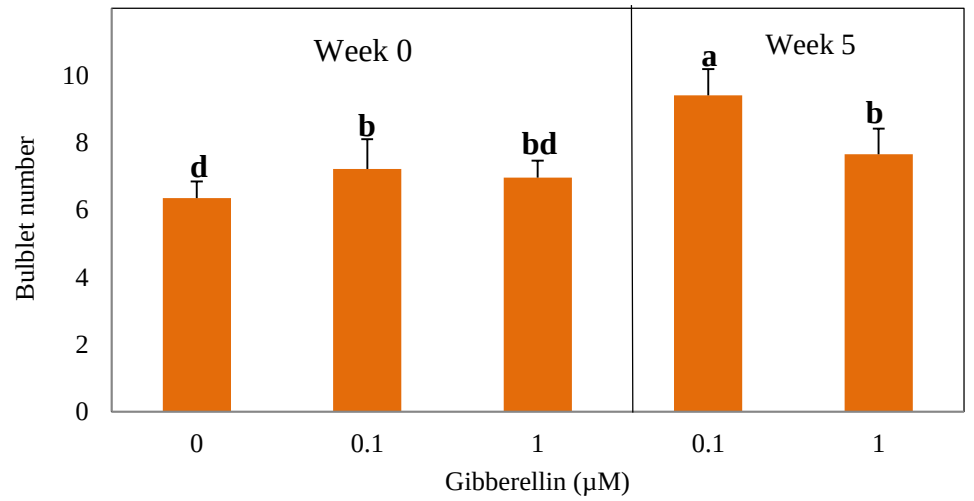

Fig. 3. Effect of different concentration of gibberellin on bulblets number. The error bar represents the standard error. The presence of letters above the error bars, as determined by Duncan's multiple range test, indicated the differences observed among the treatments (p=0.05).

The impact of gibberellin on the number of lily bulblets

Based on the findings presented in Figure 3, it is evident that the number of bulblets regenerated *in vitro* is subject to variation when exposed to different concentrations of gibberellins at two distinct application periods. The initial application of gibberellins at the commencement of the experiments did not yield any noteworthy influence on the number of bulblets regenerated when subjected to a concentration of 1 µM gibberellin. Conversely, the maximum number of bulblets was observed to increase by 50% when exposed to a concentration of 0.1 µM gibberellin, which was introduced into the medium after the fifth week (bulblet induction) (Fig. 3). The findings of this study indicate that a lower concentration of gibberellin is associated with a higher number of lily bulblets *in vitro*, while a higher concentration of gibberellin has a negative impact on lily bulblet number in both application times. Additionally, the application of gibberellin at week 5 demonstrated a positive response to the initiation of bulblets in terms of bulblet number. The findings indicated that the utilization of gibberellin during the initiation phase hindered the initiation of bulblets.

The impact of gibberellin on the growth of lily leaves

According to the data presented in Figure 4, the application of gibberellin at the initiation stage as well as at week 5 (induction stage) resulted in an increase in the fresh weight of lily leaves. However, it was observed that higher concentrations of gibberellin did not have any significant effect on the growth of lily leaves, regardless of the application time. Notably, the greatest increase in fresh weight of leaves was observed when 1 µM gibberellin was applied at week 5, exhibiting a 38% increase compared to the control (Fig. 4). Furthermore, the application of gibberellin at week 5 also led to a 20% increase in the growth of lily leaves compared to its application at the beginning of the culturing period. The findings of this study indicate that the application of gibberellin after the fifth week (induction stage) resulted in a more substantial increase in fresh leaf weight compared to applying the hormone at the beginning of the experiment. Furthermore, in the case of lily leaves, a higher concentration of gibberellin did not have a significant impact on their growth when compared to a lower concentration, regardless of the application time.

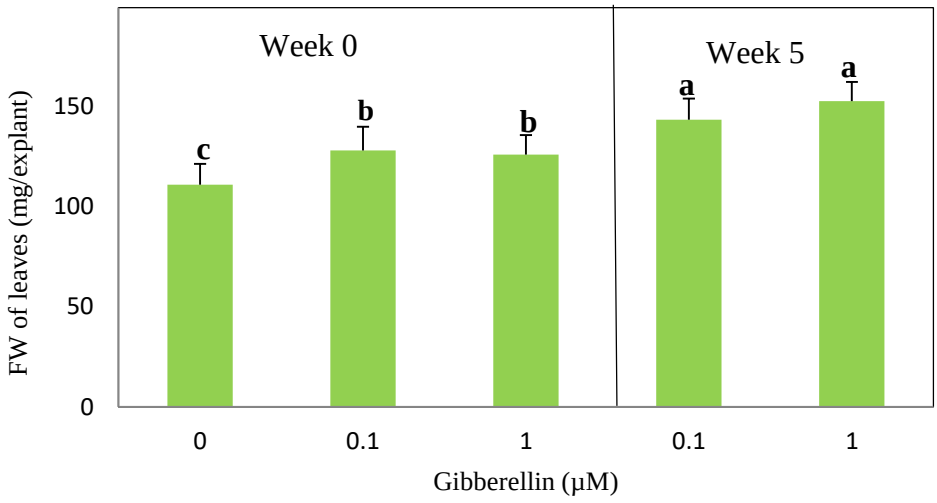

Fig. 4. Effect of different concentrations of gibberellin on FW (fresh weight) of leaves. The error bar represents the standard error. The presence of letters above the error bars, as determined by Duncan's multiple range test, indicated the differences observed among the treatments (p=0.05).

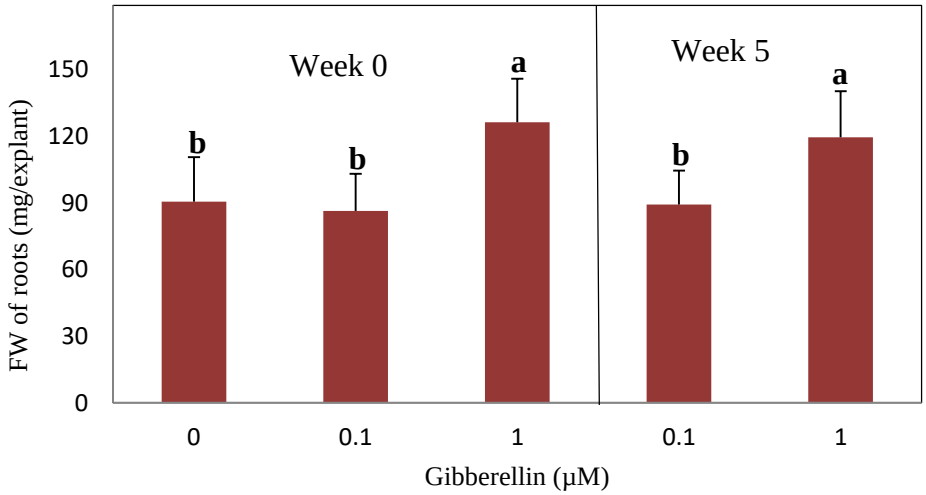

Fig. 5. Effect of different concentrations of gibberellin on FW (fresh weight) of roots. The error bar represents the standard error. The presence of letters above the error bars, as determined by Duncan's multiple range test, indicated the differences observed among the treatments (p=0.05).

Effect of gibberellin on lily root growth

The findings depicted in Figure 5 indicate that the fresh weight of the roots was solely influenced by elevated concentrations of gibberellin in both application periods. Conversely, lower concentrations of gibberellin did not enhance the growth of lily roots in comparison to the control in both application periods. Notably, the application of higher concentration of gibberellin resulted in a 40% and 32% increase in root growth when 1 µM of gibberellin was added at the initiation stage and at the fifth week, respectively (Fig. 5). The findings of these experiments indicate that the application of gibberellin at the onset of the experiment resulted in superior growth of lily roots. Specifically, the highest level of root growth (126.4 mg/explant) was observed when a concentration of 1 µM gibberellin was administered at the beginning of the experiment. It is important to note that there was no significant difference in root growth at a concentration of 1µM gibberellin, when it was applied initially or at week 5.

Effect of gibberellin on lily scale explants growth

The present study observed a reduction in the growth of scale explants under higher concentrations of gibberellin during both application times, as depicted in Figure 6. Conversely, at lower concentrations of gibberellin, no significant difference in the growth of explants was observed in comparison to the control at both application times. The findings of this study demonstrate the adverse impact of gibberellin on the growth of scale explants during lily bulblets regeneration *in vitro* at both application times.

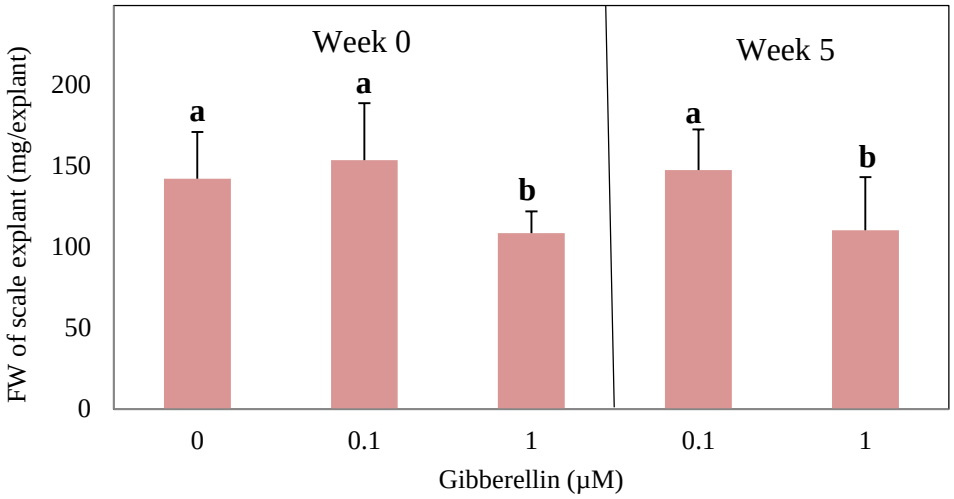

Fig. 6. Effect of different concentrations of gibberellin on FW (fresh weight) of scale explant. The error bar represents the standard error. The presence of letters above the error bars, as determined by Duncan's multiple range test, indicated the differences observed among the treatments (p=0.05).

DISCUSSION

Gibberellin is a phytohormone that plays a crucial role in regulating various developmental processes in plants, such as stem elongation, germination, dormancy, flowering, and enzyme induction (Hedden & Sponsel, 2015). Gibbrellin is a member of a major group of plant hormones that encompass around 136 distinct molecular structures., each with a skeleton of 19-20 carbon atoms (He et al., 2020). Gibberellins act as plant growth regulators by facilitating cell elongation, promoting plant height, and playing significant roles in germination, stem elongation, fruit ripening, and flowering (Shah et al., 2023). Gibberellic acids are primarily employed to stimulate the development of plantlets from adventitious embryos generated in culture (Cai et al., 2022). Nevertheless, their application is limited to certain tissue culture procedures, as they can exhibit inhibitory effects on certain plant species (Atif et al., 2021). Specifically, while gibberellins are crucial for promoting normal callus growth, they can impede organ development, such as root and shoot formation, as well as the process of somatic embryogenesis, in certain plants (Zdravković-Korać et al., 2023). In a study conducted by Le Guen-Le Saos et al. (2002), it was discovered that ancymidol, flurpirimidol, and paclobutrazol, which are known inhibitors of gibberellin biosynthesis, have the ability to stimulate bulb formation and enhance the percentage of bulbing in shallots. Meanwhile, in the context of *Hippeastrum* tissue culture, the presence of flurpirimidol not only influenced the rate of propagation but also had an impact on the size of the newly formed bulblets. More specifically, when explants were cultured in media containing flurprimidol, the bulblets were observed to be larger in size (Ilczuk et al., 2005). The findings of our study demonstrate that the addition of gibberellin to the medium significantly enhanced the growth of lily bulblets (91%), particularly when introduced after a period of 5 weeks (bulblet induction), at a concentration of 1 µM. Conversely, when gibberellin was added to the medium at the onset of the experiment (bulblet initiation), the growth of lily bulblets did not exhibit any noteworthy differences (26%) compared to the control. Furthermore, similar to the growth pattern observed in lily bulblets, both the number of lily bulblets and the fresh weight of leaves displayed comparable trends in response to the application of gibberellin. The

number of bulblets experienced a rise upon the introduction of gibberellin (0.1 µM) to the growth medium subsequent to the induction stage. However, it is noted that a higher concentration of gibberellin exhibited an adverse effect on the number of bulblets in comparison to a lower concentration. This suggests that gibberellin may have an inhibitory impact on the initiation of bulblets, but subsequently does not exert inhibitory effects on the *in vitro* bulblet induction of lily. In contrast, the response of root and scale explants to gibberellin exhibited divergent patterns of growth. Root growth was stimulated by higher concentrations of gibberellin, whereas the growth of scale explants was enhanced by lower concentrations of gibberellin, irrespective of the timing of gibberellin application. The findings of this study suggest that the application of gibberellin at various developmental stages (initiation and induction) can have a diverse impact on the growth of lily organs. Phytohormones appear to fulfill disparate functions when they are present during distinct stages of plant development. For instance, GA_3 is widely acknowledged as an inhibitor of adventitious root (AR) formation in cutting propagation (da Costa et al., 2013). However, a recent investigation on Bougainvillea uncovered that endogenous GA_3 levels experienced an initial decrease during AR development, followed by an increase during the induction and initiation stages, and ultimately a decrease during the expression stage. The researchers postulated that an elevated GA content could impede cell divisions in the early phase of rooting culture, consequently hindering the differentiation and formation of AR. Conversely, GA could enhance the elongation and growth of AR. (Huang et al., 2022). In the case of gladiolus, previous research conducted through *in vitro* studies has demonstrated that the presence of gibberellic acid can either hinder or have no impact on the formation of corms (Dantu & Bhojwani, 1995). Another study conducted on narcissus found that the application of gibberellic acid resulted in a reduction in both the quantity and weight of bulblets produced through twin-scaling (Tang et al., 2020). In the case of lycoris, the introduction of exogenous GA_3 (gibberellic acid) significantly impeded the propagation coefficient and weight of bulbs, with this inhibitory effect becoming more pronounced as the concentration of GA_3 increased (Xu et al., 2021). The inhibitory influence of gibberellin on bulblet formation of various crops has been reported while GA was administered simultaneously during the initiation and induction phases of bulblet regeneration *in vitro*, and there is no available evidence regarding the precise impact of gibberellin indivitually on either the initiation or induction stage. However, the current study presents the initial findings of utilizing gibberellin specifically on either the initiation or induction stage of lily bulblet development. Additional research is necessary to fully understand the effect of gibberellin on the different stages of lily bulblet regeneration *in vitro*.

CONCLUSION

The application of gibberellin to the growth medium in lily bulblet induction stage has been found to enhance the number of initiated bulblets and the growth of lily bulblets *in vitro*. It has been observed that various organs of the lily showed different response to gibberellin concentration. Specifically, higher concentrations of gibberellin have been found to promote the growth of lily bulblets and roots, while leaf growth does not respond positively to higher concentration of gibberellin. Conversely, the growth of scale explants is terminated by higher concentrations of gibberellin. Finally, the application of gibberellin at the initiation and induction stages individually exhibited diverse effects on the regeneration of lily bulblets *in vitro*.

Conflict of interest

The author has no conflict of interest to report**.**

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