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# *IN VITRO* MORPHOGENESIS OF GYPSOPHILA: RESPONSE TO EXOGENOUS PHYTOHORMONES

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**Abstract**

The success of *in vitro* cultivation of plant species depends on factors associated with the induction and control of morphogenesis regarding the regeneration of shoots and roots in the organogenesis process, such as the culture medium composition. Thus, the objective of this study was to investigate if the concentration of growth regulators in culture medium interferes with the *in vitro* morphogenesis of gypsophila. 'Golan' cultivar was subjected to three culture media (M), which constituted the study treatments: M1) Murashige and Skoog (MS) medium without the addition of growth regulators; M2) MS + 1 mg $L^{-1}$ of benzylaminopurine (BAP) + 0.05 mg $L^{-1}$ of naphthaleneacetic acid (NAA); M3) MS + 0.05 mg $L^{-1}$ BAP + 1 mg $L^{-1}$ NAA. After 45 days, the multiplication rate, plant height and root length were evaluated. The results showed that plantlets produced in M2 medium had a higher multiplication rate. Also, plantlets produced in M1 and M3 media showed higher shoot height and root length. It is concluded that there is a difference in the *in vitro* morphogenesis of gypsophila according to the concentration of growth regulators in the micropropagation medium.

**Keywords:** *Gypsophila paniculata* L.; growth regulators; micropropagation.

# MORFOGÊNESE DE GYPSOPHILA *IN VITRO*: RESPOSTA A FITORMÔNIOS EXÓGENOS

**Resumo**

O sucesso do cultivo *in vitro* de espécies vegetais depende de fatores associados à indução e ao controle da morfogênese quanto à regeneração de brotos e raízes no processo de organogênese, a exemplo da composição de meio de cultura. Assim, o objetivo do estudo foi investigar se a concentração de reguladores de crescimento em meio de cultura interfere na morfogênese *in vitro* de gipsofila. A cultivar 'Golan' foi submetida a três meios de cultura (M), que constituíram os

tratamentos do estudo: M1) meio Murashige e Skoog (MS) sem adição de reguladores de crescimento; M2) MS + 1 $mg.L^{-1}$ de benzilaminopurina (BAP) + 0.05 $mg.L^{-1}$ de ácido naftalenoacético (ANA); M3) MS + 0.05 $mg.L^{-1}$ BAP + 1 $mg.L^{-1}$ de ANA. Após 45 dias avaliou-se a taxa de multiplicação, altura de plantas e comprimento de raízes. Os resultados mostraram que plântulas produzidas no meio M2 tiveram maior taxa de multiplicação. Ainda, plântulas produzidas nos meios M1 e M3 apresentaram maior altura de parte aérea e comprimento de raízes. Conclui-se que há diferença na morfogênese *in vitro* de gipsofila de acordo com a concentração de reguladores de crescimento em meio de cultura na micropropagação.

**Palavras-chave:** *Gypsophila paniculata* L.; micropropagação; reguladores de crescimento.

## Introduction

Gypsophila (*Gypsophila paniculata* L.), also known as baby's breath, panicled baby's breath, or common gypsophila, is a perennial plant frequently cultivated as an annual flowering crop (GEVRENOVA *et al.,* 2010). It has its origins in the temperate regions of Europe and Asia. This versatile plant can be grown in gardens and is highly valued for its application as a filler or bedding plant in flower bouquets. It is widely recognized as one of the most prized cut flowers (WANG *et al.,* 2013; MOHAMED *et al.,* 2023). In addition, Shun *et al.* (2011) indicated that chemical compounds present in the roots of this species, such as triterpenoid saponins, allow it to be used in medicines, detergents, adjuvants and cosmetics. There are many cultivars commercially available for producers, such as the 'Million Stars', 'New Love', and 'Perfecta' (LI *et al.*, 2020).

As it is considered a genetically segregating species, the sexual propagation of gypsophila results in uneven plants (CASTRO *et al.,* 1993). The limited ability of its terminal cuttings to develop roots hinders its vegetative propagation. As a result, plant tissue culture is utilized for the commercial propagation of *G. paniculata* L. Micropropagation offers an effective and cost-efficient approach for large-scale production of disease-free, genetically identical plants suitable for the commercial cultivation of the *G. paniculata* L. plant (RADY, 2006; MOHAMED *et al.*, 2023).

The technique of rooting cuttings can cause phytosanitary problems, which are introduced into the cultivation areas through contaminated propagating material (QUOIRIN *et al.*, 2008). The plant can be contaminated by the bacterium *Pantoea agglomerans* pv. *gypsophilae* (formerly *Erwinia herbicola*), which causes galls (NISSAN *et al.*, 2006). This disease can cause significant losses of gypsophila in commercial fields and can be the limiting factor for plant propagation (MILLER *et al.*, 1981).

On the other hand, the tissue culture technique in *G. paniculata* L., based on the explants totipotentiality, has been used mainly for micropropagation and elimination of diseases (TORRES

*et al.*, 1998). Micropropagation, a technique that consists of *in vitro* vegetative propagation, is an important tool for the recovery and conservation of germplasm (DELGADO-SANCHEZ *et al.*, 2006). This technique provides maintenance of heterosis, rapid multiplication in reduced time and physical space and generation of pathogen-free plantlets (CHERUVATHUR *et al.*, 2015).

Generally, the factors that determine the success of the micropropagation of plant species are the source of the explant and the nutritive medium where they are cultivated (PASQUAL *et al.*, 2001). It is noteworthy that the standard culture medium used is Murashige and Skoog (MS) (MURASHIGE; SKOOG, 1962). However, there is still no specific culture medium suitable for a genus, species, hybrid or clone (MIYATA *et al.*, 2014).

Therefore, based on the assumption that the success of the *in vitro* cultivation of plant species depends on factors associated with the induction and control of morphogenesis regarding the regeneration of shoots and roots in the organogenesis process, it is essential to control the composition of the culture media. Thus, several classes of plant regulators have been used in tissue culture techniques, with emphasis on supplementing the medium with auxins and cytokinins (MORAIS *et al.*, 2014). Based on this, the objective of this study was to investigate if the concentration of growth regulators in culture medium interferes with the *in vitro* morphogenesis of gypsophila.

## Material and Methods

The experiment of micropropagation and clonal cleaning of the explants of gypsophila shoot tips, cultivar 'Golan', was carried out at the Laboratory of Plant Biotechnology of the University of Passo Fundo (UPF), in the year 2017.

### Plant Materials and Establishment of Aseptic Cultures

The mother plants were cultivated in greenhouses in the Horticultural Department of the UPF. The explants were isolated in an aseptic place. The plants stems were collected, sectioned and submitted to the disinfestation process. Disinfestation was performed by immersing the stems in a 70% alcohol solution for a few minutes and then in a 50% sodium hypochlorite solution for ten minutes. Then, the material was rinsed four times with double-distilled and sterilized water. After disinfestation, the shoot tips were isolated in a sterile environment (laminar flow chamber), with the aid of tweezers, scalpel and the aid of a stereomicroscopic magnifying glass. Subsequently, they were stored in test tubes containing isolation culture medium, consisting of MS medium with 1 mg $L^{-1}$ of benzylaminopurine (BAP) + 0.01 mg $L^{-1}$ of naphthaleneacetic acid (NAA) + 0.1 mg $L^{-1}$ of gibberellin ($GA_3$). For the multiplication process, the material was subcultured, sectioning the plants developed *in vitro* in order to obtain segments that contained buds (stem apices).

### Culture Conditions and evaluation

A multiplication of a gypsophila cultivation cycle was carried out using the propagules obtained *in vitro*, submitted to the MS culture medium, described by Murashige and Skoog (1962), with different growth regulators concentrations: M1) MS medium without addition of growth regulators; M2) MS + 1 mg $L^{-1}$ of BAP + 0.05 mg $L^{-1}$ of NAA; M3) MS + 0.05 mg $L^{-1}$ BAP + 1 mg $L^{-1}$ NAA.

The propagules, added in glass flasks containing the treatments, were kept in an oven for 45 days, with controlled temperature, humidity and light intensity. Prior to the evaluations, the percentage of contamination of the treatments was calculated. The attributes evaluated were multiplication rate (MR), shoot height (SH) and root length (RL).

**Experimental design and Statistical Analyses**

The experiment was carried out in a completely randomized design, with four replications. The data obtained were submitted to analysis of variance (Anova) and the averages of the treatments were compared by the Tukey test, at 5% error probability, with the aid of the Assistat program (SILVA; AZEVEDO, 2016).

## Results and Discussion

One of the biggest problems of *in vitro* propagation is the contamination of the culture medium by fungi and bacteria during the stages of this process (PALÚ *et al.*, 2011). In this study, there was 34% of contamination only in the M3 medium. Rashid *et al.* (2012) obtained 18% contamination in culture media for *in vitro* production of *G. paniculata* L. In general, there are four sources of contamination: the explant source, the nutrient medium, the environment and the operator (PIERIK, 1997). Contaminant microorganisms compete for nutrients and produce toxic substances, which inhibit the development of the explant, causing its loss (SOUSA *et al.*, 2007).

Considering the rate of gypsophila multiplication (Table 1), the highest number of propagules was obtained in the medium enriched with cytokinin BAP (M2) (Figure 1 and Figure 2A). This occurred because cytokinins are responsible for breaking apical dormancy and inducing proliferation of axillary buds in *in vitro* cultured propagules (JARDIM *et al.*, 2010). As cytokinins are promoters of cell division and differentiation, they are strongly related to plant biotechnology (PERES; KERBAUY, 2008), as they allow the multiplication of shoots *in vitro*, forming a large number of plantlets (PIASSI; PIASSI, 2016). The number of propagules, or the quantity of plantlets generated through *in vitro* propagation, presents several advantages in the context of plant production. A higher number of propagules allows for large-scale production in a relatively short timeframe. This is particularly beneficial for commercial nurseries, research institutions, and plant breeding programs that require a significant quantity of plants (BENELLI; DE CARLO, 2018).

**Table 1.** *In vitro* morphogenesis of gypsophila, 'Golan' cultivar.

| Culture media[1] | MR (number)[2] | SH (cm) | RL (cm) |
|---|---|---|---|
| M1 | 1.66±0.2 b | 5.63±1.2 a | 4.24±0.8 a |
| M2 | 4.00±0.9 a | 2.76±0.6 b | 1.01±0.1 b |
| M3 | 1.55±0.1 b | 4.40±1.0 ab | 3.11±0.7 a |
| Mean | 02.40 | 04.26 | 02.79 |
| CV (%)[3] | 25.70 | 24.37 | 20.70 |

Data presented as averages ± standard deviation. Averages followed by the same letter in the column do not differ significantly by Tukey's test ($p>0.05$).

[1] **M1**: MS medium without addition of growth regulators; **M2**: MS + 1 mg $L^{-1}$ BAP + 0.05 mg $L^{-1}$ NAA; **M3**: MS + 0.05 mg $L^{-1}$ BAP + 1 mg $L^{-1}$ NAA.

[2] MR: multiplication rate; SH: shoot height; RL: root length.

[3] CV: coefficient of variation.

**Figure 1.** Representative samples of treatments. **M1**: MS medium without addition of growth regulators; **M2**: MS + 1 mg $L^{-1}$ BAP + 0.05 mg $L^{-1}$ NAA; **M3**: MS + 0.05 mg $L^{-1}$ BAP + 1 mg $L^{-1}$ NAA.

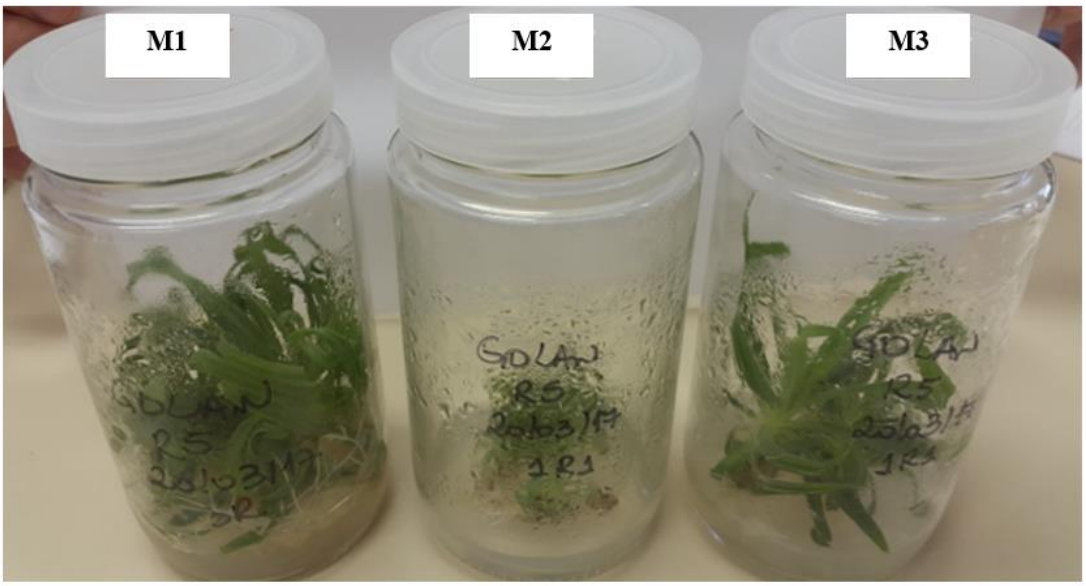
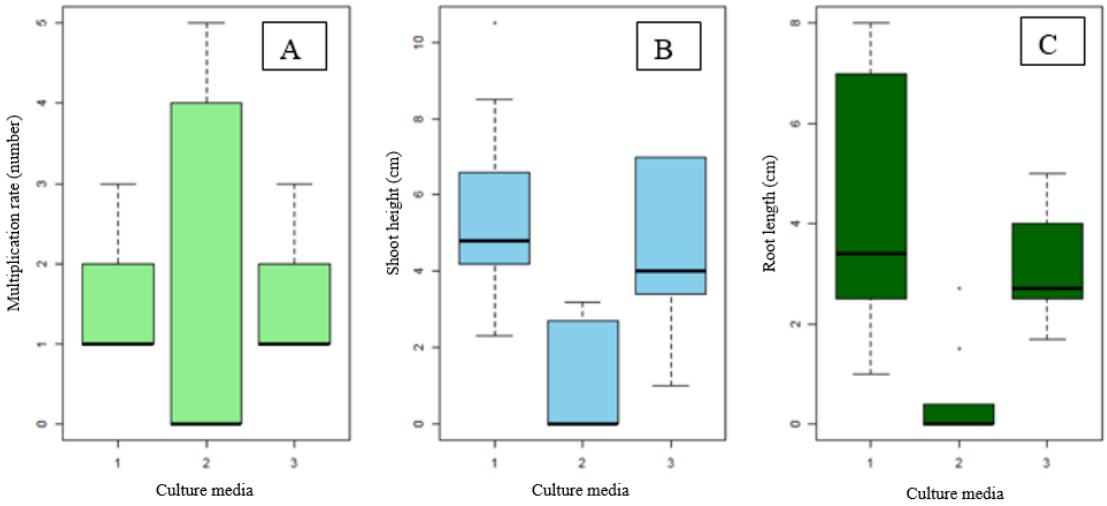


**Figure 2.** Multiplication rate (A), shoot height (B) and root length (C) according to the culture medium used in the micropropagation of gypsophila, 'Golan' cultivar. **M1**: MS medium without addition of growth regulators; **M2**: MS + 1 mg $L^{-1}$ BAP + 0.05 mg $L^{-1}$ NAA; **M3**: MS + 0.05 mg $L^{-1}$ BAP + 1 mg $L^{-1}$ NAA.




Propagules grown on M1 medium had higher shoot height compared to those grown on M2 medium, but they did not differ from propagules grown on M3 medium (Table 1). Although the M2 medium was superior to the other media in terms of shoot formation, the plant size was lower than the other treatments (Figure 2B). This was because cytokinins generally stimulate multiplication and inhibit shoot length (TAIZ *et al.*, 2017). Furthermore, it is known that the length of shoots is related to their competition for nutrients (CAMPOS *et al.*, 2007). Thus, as there was greater formation of shoots in the M2 medium, these shoots probably competed more for nutrients, causing a reduction in the height of the aerial part of the plantlets. Rashid *et al.* (2012) obtained similar results, in which culture media with higher concentrations of BAP resulted in a greater number of aerial shoots. Being the best result was obtained with a 1.0 mg $L^{-1}$ concentration (RASHID *et al.*, 2012).

Ustuner *et al.* (2022) tested different culture media for the *in vitro* propagation of *G. pilulifera* from two different locations. The highest shoot length was obtained for the MS medium containing 2 mg $L^{-1}$ BAP + 2 mg $L^{-1}$ IAA (Indoleacetic acid), with averages of 10.50 ± 0.172 mm and 6.83 ± 0.17 mm, respectively. Shoot length holds several advantages in the context of *in vitro* propagation. One significant advantage is the potential for increased multiplication rates. Longer shoots often possess more nodes, which serve as potential sites for further shoot proliferation. This characteristic allows for the production of a larger number of plantlets from a single explant, significantly enhancing the efficiency of propagation efforts. Furthermore, longer shoots often exhibit enhanced

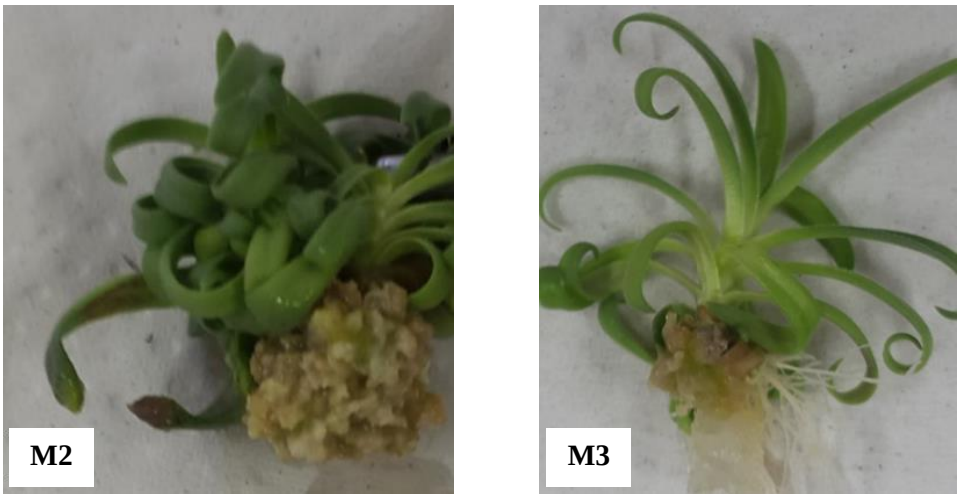
vigor and physiological maturity, which can positively influence their acclimatization and survival rates upon transfer to *ex vitro* conditions (BENELLI; DE CARLO, 2018).

Plantlets produced in M1 and M3 media had greater root lengths (Table 1) in relation to those obtained in M2 media (Figure 2C). This result corroborates previous research, which showed that the use of culture media without growth regulators, in the rooting stage, has been successful (CARVALHO *et al.*, 2013; SOUZA *et al.*, 2013) because, in some cases, the endogenous levels of auxin are sufficient to induce rooting, dispensing the exogenous application of this regulator (BOSA *et al.*, 2003). However, previous studies demonstrated that BAP and NAA in the culture media for the development of *G. paniculata* L. result in different responses on structure morphogenesis (RASHID *et al.*, 2013). These results are important when the regeneration interest is in a specific plant structure.

Abdillahi and Sezgin (2022) found the highest root count in the MS medium, and it was noticed that the combination that gave the best results was the one containing 0 mg $L^{-1}$ KIN + 0.5 mg $L^{-1}$ IBA, 1 mg $L^{-1}$ BAP + 0.25 mg $L^{-1}$ IBA. The length of the roots varies between 0.5 cm and 8 cm. Root length offers several advantages that contribute to the successful establishment and growth of plants. Firstly, longer roots provide increased surface area for nutrient absorption. This enables efficient uptake of essential minerals and water, promoting robust growth and development of the plantlets. A well-developed root system facilitates improved nutrient utilization, leading to healthier plants with higher survival rates during acclimatization and transplantation. Additionally, longer roots enhance anchorage and stability, reducing the risk of toppling or damage caused by environmental factors or handling. This is especially beneficial when plants are being transferred to *ex vitro* conditions, where a strong root system aids in the establishment and adaptation of the plants (MOHAMED *et al.*, 2023).

Induction of callogenesis was observed in plantlets produced in M2 and M3 media (Figure 3). This occurred because the hormones auxin and cytokinin act in synergism and, in intermediate balances, induce the formation of a mass of undifferentiated cells from plant tissue, called callus (PIASSI; PIASSI, 2016). Although not desirable, calluses are common in the micropropagation of gypsophila. Barakat and El-Sammak (2011) observed the formation of calluses in all protocols tested for micropropagation of *G. paniculata* L., and explants originating from shoot tip formed less calluses than lateral buds.

**Figure 3.** Callus formation in gypsophila propagules, 'Golan' cultivar, grown in M2 and M3 culture media. M2: MS + 1 mg $L^{-1}$ BAP + 0.05 mg $L^{-1}$ NAA; M3: MS + 0.05 mg $L^{-1}$ BAP + 1 mg $L^{-1}$ NAA.




## Conclusion

There is a difference in the *in vitro* morphogenesis of gypsophila according to the growth regulators concentration in the micropropagation culture medium. The culture medium with the highest concentration of BAP in relation to NAA promotes a higher number of shoots (higher multiplication rate). In media without growth regulator and with a higher concentration of NAA in relation to BAP, there is a better development of shoots.

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