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In vitro Regeneration of *Gypsophila Paniculata* L. Through Nodal Segments

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ABSTRACT

Gypsophila (*Gypsophila paniculata* L.), commonly known as baby's breath, is a very popular ornamental plant in the floriculture industry. Gypsophilas are often grown as ornamental plants in many European gardens. They are valuable as a cut flower in floristry to add as a filler to flower bouquets. A protocol for the regeneration of *Gypsophila paniculata* L. using nodal explants grown in the field was established. The induction of multiple shoots was best obtained on Murashige and Skoog (MS) medium supplemented with 1 mg L^{-1} BAP and 0.5 mg L^{-1} NAA. After 8 weeks of initial culture, multiple shoots were regenerated from the explants and transferred to the rooting medium with different concentrations of IBA and NAA. The highest number of roots was obtained from the MS medium supplemented with 0.5 mg L^{-1} IBA and 0.5 mg L^{-1} NAA within 6 weeks. Rooted plantlets were hardened and established in pots with 89% highest survival rate.

1. INTRODUCTION

Gypsophila (*Gypsophila paniculata* L.), commonly known as Baby's breath, is a very popular ornamental plant in the floriculture industry. It belongs to the family Caryophyllaceae and it is native to Europe, Asia and North Africa as an annual crop (Barakat & El-Shammak, 2011). Many species are found on calcium rich soils, including gypsum, which is depicted in the name of the genus, "gypsophila." Its botanical name means "lover of chalk," which is accurate in describing the type of soil in which this plant grows (Barakat & El-Shammak, 2011).

Gypsophilas are often grown as ornamental plants in many European gardens, and it is valuable as a cut flower in floristry to add as a filler to flower bouquets (Ayeh et al., 2009). Gypsophila is now the main flowering shoot used fresh and dried as filler in floral arrangements all over the world.

The flowers of commercial *G. paniculata* plants are sterile and do not produce seeds; therefore, breeding programs are severely restricted (Ayeh et al., 2009). Also, the low rooting frequency of vegetatively propagated cuttings disturbs propagation. Thus, there is a need to explore other more effective methods of propagation. Hence, the *in-vitro* culture techniques can be the alternative for the continuous provision of plantlet stocks for large scale field cultivation. Gypsophila tissue culture has been used for micropropagation and viral elimination (Han et al*.,* 1991). Works by Kusey et al*.* (1980), Pauthe- Dayde et al. (1990) and Henry (1993) have dealt with multiplying shoot cultures but stop short of producing a reliable adventitious shoot-regeneration method, an absolute prerequisite for establishing an efficient *in vitro* mutagenesis procedure.

The aim of this study was to improve the different stages of *in-vitro* propagation of *Gypsophila* to allow commercial production of this plant in Sri Lanka, through modification of culture media for initiation of shoots, shoot proliferation, and rooting of shoots.

2. MATERIALS AND METHODS

Healthy and young shoot cuttings of *G. paniculata* bearing five to eight nodes were collected from four months old, donor plant growing in plant house of Uva Wellassa University of Sri Lanka. The nodal segments (1.0 – 1.5 cm) were thoroughly washed under running tap water for 1 hour. Then explants were surface sterilized with 5% NaOCl and tween 20 (10 min), 5% NaOCl (5 min) with three successive washing with double distilled water. Under aseptic condition, nodal segments were surface sterilized with 70% ethanol for 1 minute and finally washed three times with sterilized water.

MS medium (Murashige and Skoog, 1962) and vitamins supplemented with 30 g L^{-1} sucrose were used as the basal medium. Details of the MS with different hormonal combinations used for shoot multiplication in *G. paniculata* are given in Table 1. Each jar with four explants was considered as a replication. Four replications were used for each medium protocol. S_0 medium was used as the control.

Cultures were maintained at 25±1°C air temperature in a culture room with a 16/8h light/ dark photoperiod. Photosynthetic photon flux intensity, 40 µmol m^{-2} s^{-1} provided by cool-white, fluorescent lights. Eight weeks after introducing to each medium, mean number of shoots and mean length of the shoot were calculated.

After eight weeks of incubation, newly emerged shoots were introduced to jars containing MS medium with different growth regulators for root induction as mentioned in Table 2. Four replications with three nodal segments were used for each medium and R_0 medium considered as the control treatment. Culture bottles were

incubated as previously described for six weeks for root formation. Number of roots per shoot and average length of the shoot were recorded after six weeks.

After developments of shoots and roots, two successive washes were done for regenerated plants with lukewarm water to remove agar from the roots and then with Captan™ fungicide to avoid fungal attacks after planting on the medium. These well washed plants were transplanted to small polythene pots filled with sterilized coir dust and sand (3:1 v/v). Ten repetition and one plant in each repetition have used for the acclimatization stage. The pots were incubated in the growth chambers for acclimatization. After 2 weeks of hardening, mortality rate was determined as a percentage.

The data were analysed using ANOVA in Complete Randomized Design (CRD) with four replicates. Minitab 16.1 computer package was used to analyze the data and the difference between the mean values were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

3. RESULTS

The ability to regenerate large number of shoots from cultured tissues is important for the success of large-scale commercial production of *Gypsiphila* floriculture industry.

In the present study, most of the nodal explants produced shoots directly on the shoot induction media and few were given calluses on the media. After eight weeks of period nodal explants cultured on MS medium with several combinations of BAP and NAA, developed multiple shoots. Apical buds were turned into dark green at the end of the shoot initiation stage and emerging shoots were observed at the base of the nodal explants. Number of shoots initiated, and the length of the emerged shoots were measured, after eight weeks culturing to determine best hormonal combination for shoot initiation and subsequent growth. During the shoot initiation process, few replicates showed the vitrification phenomenon.

3.1 NUMBER OF SHOOTS PER EXPLANT

Results obtained revealed that the medium consisting with 1 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (S_3) recorded the highest number (31) shoots for four explants in the culture bottle with green expanded leaves. There was a significant difference ($p \leq 0.05$) in shoot number per explant in different treatment combinations after eight weeks of shoot induction. Number of 27, 19 and 18 shoots were recorded in treatment S_8 (0.5 mg L^{-1} BAP and 0.25 mg L^{-1} NAA), S_2 (1 mg L^{-1}BAP and 0.25 mg L^{-1} NAA) and S_6 (0.75 mg L^{-1} BAP and 0.5 mg L^{-1} NAA) respectively (Table 01).

3.2 SHOOT LENGTH

There was a significant difference ($p \leq 0.05$) of shoot length in different treatment combinations after eight weeks of shoot induction. Incorporating 1 mg L^{-1} BAP and 0.5 mg L^{-1} NAA (S_3) to the MS basal media showed highest plant length (22 mm) compared to the control treatment. Next to the S_3 treatment S_8 (0.5 mg L^{-1} BAP and 0.25 mg L^{-1} NAA), S_1 (1 mg L^{-1} BAP) and S_5 (0.75 mg L^{-1} BAP and 0.25 mg L^{-1} NAA) treatments gave the highest shoot length of 21 mm, 20.7 mm and 20.4 mm respectively (Table 01).

3.3 NUMBER OF ROOTS PER SHOOT

Roots that developed on this medium were thick, long and fibrous, six weeks after culturing on the medium. The effect of IBA and NAA concentration on the forming roots number of *G. paniculata* shoots is mentioned in Table 02. There was a significant difference ($p \leq 0.05$) in root number per shoot in different treatment combinations after six weeks of root induction. Highest number of roots per shoot was obtained from the medium

consisting of 0.5 mg L^{-1} IBA and 0.5 mg L^{-1} NAA (R_6) with 9 roots. Lowest number of roots (3 roots) were given by the R_2 (0.5 mg L^{-1} IBA and 0.25 mg L^{-1}NAA), R_5 (0.25 mg L^{-1} IBA and 0.5 mg L^{-1} NAA) and control treatment.

3.4 ROOT LENGTH

Incorporating 0.5 mg L^{-1} IBA and 0.5 mg L^{-1} NAA (R_6) showed the highest root length (85 mm) in the rooting medium. 74 mm and 59 mm of root length were the second and third highest root lengths given by the R_{11} (0.75 mg L^{-1} IBA and 0.75 mg L^{-1} NAA) and R_8 (1 mg L^{-1} IBA and 0.5 mg L^{-1} NAA) respectively. Hence, there was a significant difference ($p \leq 0.05$) in root length in different treatment combinations after six weeks of root induction (Table 02).

3.5 PLANTLET ACCLIMATIZATION

The survival rate of the plants was 89%. After 14 days the plants were shifted to the greenhouse and after another two to three weeks, the plants were successfully transformed to fields.

4. DISCUSSION

Most of the previous studies on *G. paniculata* have focused on developing adventitious shoot regeneration from internodes or leaf segments (Barakat et al., 2011; Kanchanapoom et al., 2011; Ayeh et al., 2009). Moreover, callus and cell suspension cultures were initiated from leaf segments were reported by Ayeh et al., in 2009. Kanchanapoom et al., 2011 obtained plantlets derived from callus grown in the MS medium supplemented in 44.3 µM BA.

In general, high or low concentration of BA (6- Benzyl amino purine) with NAA documented in lower number of shoot and lower values for shoot length (Ayeh et al., 2009). The harmonious effect of NAA in combination with BA on promotion of *G. paniculata* shoot cultures is in agreement with observations of Ayeh *et al*., (2009) who obtained regenerated plants from shoot tips of *Gypsophila paniculata.* The MS medium supplemented with 0.5 mg L^{-1} BA + 0.5 mg L^{-1} NAA + 1.0 mg L^{-1} Kinetin which resulted a high proliferation rate (10.4 shoots) was documented by Barakath et al. in 2011. Ayeh et al., (2009) and other several researchers have reported that BAP, Kinetin, NAA and IBA in combination of each other prove the best media for micropropagation or *in vitro* growth of *Gypsophila* plant. In Han et al., (2009), found that 0.2 mg L^{-1} BA + 0.1 or 0.2 mg L^{-1} NAA is resulted the best performances for *in vitro* shoot tip proliferation of *Gypsophila paniculata* L. In 1991(a) Han et al., also recorded that BA at 0.5 - 2 mg L^{-1} was the most effective cytokinin level for *Gypsophila paniculata* shoot proliferation and each at 0.1 - 0.3 mg L^{-1} combination of BA and IAA resulted best shoot proliferation and growth than BA alone.

Rashid et al., 2012 reported that the MS medium containing 0.5mg L^{-1} of NAA gave the best result (85%) for the root formation. And also, Ayeh et al, (2009) and Zuker et al., (1997) reported the NAA as the best medium for root development of Gypsophila plant.

5. CONCLUSION

However, our study at present reveals that two growth regulators at moderate concentrations produced best performances in shoot induction *i.e* 1 mg L^{-1} BAP and 0.5 mg L^{-1} NAA during moderate time periods compared to most of the previously reported works. There is less reported data on rooting of Gypsophila, but we obtained better rooting with 0.5 mg L^{-1} IBA and 0.5 mg L^{-1} NAA during six weeks of period. Findings related to root development in our study is on par with findings of Ayeh et al., (2009) and Zuker et al., (1997) who reported that NAA as the best medium for root development of Gypsophila.

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Table 01: Effect of different concentrations of BAP & NAA on *invitro* shoot regeneration & shoot length of *G. paniculate*

Treatment	Hormonal Combination		Number of Shoots	Shoot Length (mm)
	BAP (mg L^{-1})	NAA (mg L^{-1})		
S_1	1.00	0.00	16.31 ± 3.308 ^{cde}	20.725 ± 0.286 ^{a}
S_2	1.00	0.25	19.57 ± 3.308 ^{c}	20.441 ± 0.479 ^{b}
S_3	1.00	0.50	31.07 ± 1.261 ^{a}	22.171 ± 0.955 ^{b}
S_4	0.75	0.00	13.16 ± 0.870 ^{ef}	18.513 ± 1.320 ^{ab}
S_5	0.75	0.25	16.00 ± 0.816 ^{de}	20.454 ± 0.335 ^{b}
S_6	0.75	0.50	18.11 ± 1.071 ^{cd}	19.794 ± 0.128 ^{bc}
S_7	0.50	0.00	11.57 ± 0.948 ^{gf}	19.954 ± 0.417 ^{b}
S_8	0.50	0.25	27.50 ± 1.192 ^{b}	21.099 ± 0.120 ^{c}
S_9	0.50	0.50	14.71 ± 0.617 ^{def}	14.985 ± 0.313 ^{d}
S_0 (Control)	0.00	0.00	9.61 ± 1.542 ^{g}	12.719 ± 0.213 ^{e}

* Means ± SD within the same column followed by different letters are significantly different at $p< 0.05$

Table 02: Effect of different concentrations of IBA & NAA on *invitro* root regeneration & root length of *G. paniculata*

Treatment	Hormonal Combination		Number of Roots	Root Length (mm)
	IBA (mg L^{-1})	NAA (mg L^{-1})		
R_1	0.25	0.25	6 ± 0.8165 ^{bcde}	51.75 ± 4.5 ^{cd}
R_2	0.50	0.25	2.75 ± 0.5 ^{g}	38 ± 8.165 ^{de}

R_3	0.75	0.25	6.5 ± 0.577 ^{bcd}	50.25 ± 15.283 ^{cd}
R_4	1.00	0.25	5 ± 0.816 ^{cdef}	43 ± 3.830 ^{de}
R_5	0.25	0.50	2.75 ± 0.500 ^{g}	33.5 ± 1.291 ^{ef}
R_6	0.50	0.50	8.75 ± 0.957 ^{a}	85 ± 4.454 ^{a}
R_7	0.75	0.50	4.5 ± 0.291^{defg}	45 ± 3.559 ^{cde}
R_8	1.00	0.50	4.5 ± 0.291 ^{defg}	59.25 ± 13.647 ^{bc}
R_9	0.25	0.75	3.5 ± 0.577 ^{fg}	53 ± 4.163 ^{cd}
R_{10}	0.50	0.75	7 ± 0.817 ^{abc}	42.5 ± 1.291 ^{de}
R_{11}	0.75	0.75	7.25 ± 0.957 ^{ab}	74 ± 2.161 ^{ab}
R_{12}	1.00	0.75	6.75 ± 0.500 ^{abc}	33 ± 2.708 ^{ef}
R_{13}	0.25	1.00	3.5 ± 0.577 ^{fg}	50.5 ± 5.196 ^{cd}
R_{14}	0.50	1.00	4 ± 0.000 ^{efg}	44.75 ± 1.258 ^{cde}
R_{15}	0.75	1.00	7 ± 0.817 ^{abc}	52.75 ± 0.957 ^{cd}
R_{16}	1.00	1.00	5.25 ± 0.957 ^{bcdef}	50.5 ± 5.745 ^{cd}
R_0(Con-trol)	0.00	0.00	2.5 ± 1.291 ^{g}	20.75 ± 2.062 ^{f}

* Means ± SD within the same column followed by different letters are significantly different at $p< 0.05$