Annals of the Sri Lanka Department of Agriculture 2012. 14: 125-133.

A SIMPLE MASS PROPAGATION PROTOCOL FOR BABY'S BREATH (*Gypsophila paniculata* L.)

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ABSTRACT

The study describes a simple, low cost, mass-scale planting material production method for Baby's breath (*Gypsophila paniculata* L.), which is a popular filler in different types of floral arrangements. *In vitro* multiplication was successfully performed on MS medium with 1 mg L^{-1} BAP. Variation in the response was observed in the rate of multiplication through single nodal cuttings. Out of the two accessions tested, accession 1 had a multiplication rate of 27.47 per sub culture while accession 2 showed a rate of 19.19 per subculture. The study was more focused on *ex vitro* rooting of microshoots using locally available low cost materials in a three factor factorial experiment. The results revealed that the percentage of rooted plants and survival rates of the microshoots were higher in sand alone and in the mixture of coir dust and sand, compared to the soil-based rooting medium. The microshoots raised in the sand medium had higher number of roots and recorded the maximum fresh weight at five weeks after transferring, in both accessions. The treatment combinations of the accession and age of plants also affected the number of roots and fresh weight of plants. The accession 2 produced a statistically similar number of roots ($p>0.05$) regardless the age, and the highest fresh weight (0.42 g) was also recorded in five weeks old plants of the same accession. However, statistically different number of roots ($p<0.05$) was produced by the plants of accession 1 across the three age levels. Both accessions showed the maximum plant height in the sand medium and the highest fresh weight was recorded by the plants of accession 2 indicating the potential of sand as a medium for *ex vitro* rooting. These plants exhibited almost 100 % plant survival when transferred to poly bags containing a mixture of top soil sand and organic matter at the ratio of 1:1:1.

KEYWORDS: *Ex vitro* rooting, *Gypsophila paniculata*, *In vitro* multiplication

INTRODUCTION

Baby's breath (*Gypsophila paniculata* L.) is a perennial plant, often grown commercially as an annual crop. It is valued as a cut flower in floristry and added as a filler to flower bouquets and several other floral arrangements. Baby's breath is mainly grown in the upcountry areas where the climate favors growing of a vast array of floricultural species from diverse origins. Conventional propagation of this species is done through tip cuttings and it is hindered by the low rooting percentage. Thus, micropropagation is important as an efficient alternative propagation method to assure the continuous supply of plantlets for large scale field cultivation. *In vitro* culture techniques are widely used to propagate many floricultural crops and *G. paniculata* is a species amenable for *in vitro* multiplication. Micropropagation of

Gypsophila involves usage of shoot tips as explants, which produce adventitious shoots with the application of NAA and BAP as growth regulators (Rady, 2005; Ayeh *et al.*, 2009; Barakat and El-Sammak, 2011).

In vitro rooting of micropropagated shoots is often accomplished in agar-solidified media. Upon completion of the rooting period, the medium should be removed from the roots which are usually very weak (Hazarika, 2006) to avoid microbial infections during the acclimatization period. This exercise is laborious and roots can further be damaged when handling them improperly. Further, the roots produced *in vitro* are without root hairs (Hazarika, 2006). Therefore, during the early acclimatization period, the roots do not function normally to support the plants as anchors and to uptake water and nutrients. In addition, plantlets without roots are much easier and faster to be planted on a medium than those with roots. In most cases acclimatization is a difficult phase in micropropagation. It is necessary to provide optimal environmental factors in order to accomplish the gradual transition of the plantlets from *in vitro* environment to the conditions of the natural environment. This situation necessitates a simple plant propagation technology and *ex vitro* rooting can be proposed as a viable low cost approach.

Ex vitro rooting of microshoots has been applied in micropropagation of various woody perennial plants (de Klerk, 2002). The method reduces the per plant costs as it waives off the need for asepsis and specialized handling, and leads to simultaneous hardening of plantlets (Debergh and Maene, 1981). Therefore, in the present study, two accessions of Baby's breath were propagated *in vitro* and rooted under *ex vitro* conditions using locally available low cost materials in order to develop a simple low cost mass propagation method for the species.

MATERIALS AND METHODS

Plant material

Shoot tips of two distinct accessions (accession 1 and accession 2) of *Gypsophila paniculata* L., collected from polytunnel-grown plants in Nuwara Eliya, Sri Lanka were used as the plant materials and the experiment was carried out at the Agricultural Research Station at Sita Eliya, Nuwara Eliya.

In vitro multiplication

Shoot tips were washed with 2-3 drops of a liquid detergent under running tap water for 20 min and subsequently surface sterilized with 70 % alcohol for 30 sec followed by 20 % Clorox for 12 min and then rinsed 3 times with sterilized distilled water in a laminar flow bench. Shoot tips were dissected and 1-2 cm long explants were established on solid MS medium (Murashige and Skoog, 1962) supplemented with 0.2 mg L^{-1} BAP and 0.02 mg L^{-1} NAA in culture tubes as 1 shoot per tube. After 3 weeks, the shoots were transferred to MS medium supplemented with 1 mg L^{-1} BAP + 7 g of agar (Meron, Bacteriological superfine) + 30 g of ordinary white sugar for shoot multiplication. All the cultures were incubated under light at 2500 lux for a period of 16 hrs per day at 25 ± 1°C. *In vitro* multiplication was performed through nodal cuttings at every 3-4 weeks up to 4 cycles on 20 ml of the above stated medium in half size jam jars, with 3 explants in each jar. The subculture period (3-4 weeks) was determined as suggested by Ayeh *et al.* (2009) to avoid vitrification or hyperhydricity which is a common phenomenon in tissue culture of *Gypsophila*. The number of shoots and total number of nodal cuttings at each subculturing were recorded.

Ex vitro rooting of microshoots

The tip cuttings measuring 3-4 cm in length were dissected from the *in vitro* regenerated shoots and planted in the rooting media *viz,* (1) sand (coarse sand), (2) coir dust:sand 3:1 and (3) sand:subsoil:organic matter 1:1:2., which were steam sterilized for 3 hrs. The rooting media were filled in 51 *x* 30 cm trays with 24 numbers of 6.5 cm diameter cups and each cup held 5 microcuttings. Before planting, a pinch of ground Triple Supper Phosphate was mixed to each cup. The nursery trays were shifted to a propagator after planting and 5 ml of Albert solution (5 g L^{-1}) solution was applied at the end of the third week and further maintained up to five weeks. The average temperature inside the propagator was in the range of 32-36 °C in the morning (9.00 am) and reached 35-42 °C at 12.00 noon where as the light intensity during noon ranged between 12,000-24,000 lux during the period of study.

The percentage values of plant survival and rooting as well as data on growth and rooting were recorded commencing from the third week and continued up to the fifth week. Plant growth in different media was measured by the data on shoot height and fresh weight. After date collection the plants were potted to observe the plant survival. One plug with five plants was considered as a replicate and at each week, 50 plants (10 replicates) were uprooted and used for recording of data.

The experiment was laid as a Complete Randomized Design (CRD) with three factors (accession, plant age and the rooting medium) in a factorial arrangement. The rooted plants were potted in a soil mixture and maintained inside a glass house. Data analysis was done through ANOVA using SAS (portable 9.1) computer software package and the count data were transformed using appropriate transformation methods before analysis.

RESULTS AND DISCUSSION

In vitro multiplication

The effect of the accession on the rate of proliferation is shown in Table 1. The rate of multiplication was significantly different ($p<0.05$) between the accessions.

Table 1. The average rate of *in vitro* multiplication of two different accessions of *G. paniculata* during four cycles of subculture

Accession	*Percentage of explants forming shoots*	*Mean number of shoots/plant/subculture*	*Mean number of nodal cuttings/plant/subculture*
Accession 1	100	14.86 [a]	27.47 [a]
Accession 2	100	6.76 [b]	19.19 [b]
CV %	-	10.58	5.56

Within a column, the means followed by the same letter are not significantly different at $p=0.05$ ($n = 30$)

A preliminary *in vitro* study has shown that the MS medium with 1 mg L^{-1} BAP as the only plant growth regulator can result in significant proliferation of shoots in *G. paniculata* (Anonymous, 2009). In the present study, too, a higher number of shoots per subculture (6.76 – 14.86) was achieved with BAP only. However, Rady (2005) could achieve only 4.2 nos. of shoots per subculture on MS medium supplemented with 0.5 mg L^{-1} each of NAA and BAP in *Gypsophila* and recorded 18 % vitrified shoots. Ahroni *et al.* (1997) found that thidiazuron (TDZ) as the most effective cytokinin, with up to 100 % of the explants (nodal cuttings of the stem of *G. paniculata*) forming shoots, at an average of 19 shoots per explant. In the present study, BAP was used as a cheaper alternative to TDZ, however, the same degree of shoot formation was recorded. Use of nodal segments substantially increased the rate of multiplication by 1.85-fold in accession 1 and 2.84-fold in accession 2 when compared to using shoots. The growth of accession 2 was robust and distinct during the *in vitro* stages and hence may have led to a significantly lower ($p<0.05$) number of shoots.

Ex vitro rooting

The plant survival and rooting percentages during the *ex vitro* rooting phase is shown in Table 2. Though the data were not analyzed statistically, in the sand only medium and the coir dust:sand mixture, plant survival and rooting were similar while in the soil mixture plant survival and rooting were poor.

Table 2. Effect of media on percentages of plant survival and rooting (values within parenthesis) of two accessions during *ex vitro* rooting period

Rooting medium	Percent values of plant survival and ex vitro rooting					
	Accession 1			Accession 2		
	3 WAP	4 WAP	5WAP	3 WAP	4 WAP	5WAP
Sand	95.8 (88)	95.8 (100)	94.2 (98)	98.3 (98)	97.1 (100)	97 (100)
Coir dust:sand	97.5 (68)	97.1 (90)	93.7 (92)	96.7 (98)	94.6 (100)	94 (100)
Soil-based medium	90.8 (34)	62.5 (46)	58.3 (84)	82.1 (62)	80.4 (88)	61 (90)

Andersen (1986) reported that an appropriate rooting medium should generally have an optimal volume of gas-filled pore space and an oxygen diffusion rate adequate for the needs of respiration. Rapid and higher rooting in sand recorded in the present study could be attributed to these favourable properties of sand and coir dust sand media in inducing rooting. However, accession differences were recorded in both sand and coir dust:sand media. Further, the morphological characteristics of the accession 2, especially the bigger leaves and thicker stem, would have favored a higher photosynthesis and thereby induced rooting. Mpeck and Atangana (2007) recorded a significant substrate *x* leaf area interaction on the rooting percentage and the number of roots per rooted cutting of *Baillonella toxisperma* leafy stem cuttings in a non-mist propagator. The influential effect of leaf area on rooting has also been examined for *Cordia alliodora* (Mes'en *et al*., 1997).

The poor rooting percentage of microcuttings observed on the soil medium could be due to high water content, less porosity and practical difficulties in maintaining appropriate water content. In a similar study, Ayeh *et al.* (2009) achieved 90 % plant survival with the microshoots treated with rooting hormone and grown in a mixture of perlite. The survival and rooting percentages of microshoots achieved in this experiment was comparable with the rate of plant survival in acclimatization of *in vitro* rooted *Gypsophila* plants. It was reported that only 90 % of the *G. paniculata* plants survived when rooted *in vitro* on MS with 3 mg L^{-1} IBA (Rady, 2005).

The interaction of rooting medium and the age of the plants was significant for the number of roots per plant (p=0.0001) and for fresh weight (p=0.0028) in both accessions (Table 3). Despite the age differences, the highest number of roots were recorded in plants in the sand medium followed by five and four weeks-old plants in the coir dust:sand and five weeks-old plants in the soil-based medium.

The lowest number of roots per plant was observed with three and four weeks-old plants in the soil-based medium. The soil-based rooting medium and coir dust:sand medium have higher water holding capacity when compared to sand. The soil-based medium has the better nutrition compared to the other two media, due to added organic matters. Rooting was quicker and higher in the sand medium, and the nutrient content of the medium did not influence rooting of *G. paniculata* microshoots. Sand has been effectively used for *ex vitro* rooting of sugarcane in a misting chamber by Pandey *et al.* (2011). The study revealed that sand has optimal properties for rooting. Further, sand is the most commonly used medium for the propagators, and has been identified as the best rooting medium for *Cordia alliodora* (Boraginaceae) (Leakey *et al.*, 1990), *Allanblackia floribunda* (Guttiferae) (Atangana *et al.*, 2006) and *Geranium* (Geraniaceae) (Mamba and Wahome, 2010) stem cuttings.

Table 3. Number of roots and fresh weight of plants as influenced by the treatment combinations of rooting medium and age of the plants

Treatment combination	*Mean number of roots/plant*	*Mean fresh weight (g)*
Sand *x* 4 weeks	2.75 [a]	0.28 [b]
Sand *x* 5 weeks	2.54 [ab]	0.38 [a]
Sand *x* 3 weeks	2.47 [abc]	0.16 [c]
Coir dust:sand *x* 5 weeks	2.14 [bc]	0.29 [b]
Coir dust:sand *x* 4 weeks	2.09 [bc]	0.14 [c]
Coir dust:sand *x* 3 weeks	1.95 [c]	0.11 [cd]
Soil-based *x* 5 weeks	1.99 [c]	0.29 [b]
Soil-based *x* 4 weeks	1.28 [d]	0.12 [cd]
Soil-based *x* 3 weeks	1.04 [d]	0.08 [d]
CV %	14.95	27.8

Within a column, the means followed by the same letter are not significantly different at p=0.05

Table 3 also shows the variation of fresh weight with the different treatment combinations of medium and plant age. While the five weeks-old plants in sand medium recorded the highest fresh weight (0.38 g $plant^{-1}$), which is significantly higher ($p<0.05$) than all other combinations, three weeks-old plants in the soil-based medium recorded the least fresh weight (0.08 g $plant^{-1}$). The fresh weight data, however, did not show a strong relationship with the number of roots.

The interaction between the accession and age of the plants was significant for the number of roots (p=0.0001) and for plant fresh weight (p=0.0001) (Table 4). Statistically similar high number of roots was produced by both accessions in the five weeks-old plants, and in three weeks-old plants of accession 2. Table 3 showed that three weeks time period was sufficient for microcuttings to be rooted in a sand medium for both accessions.

Table 4. Number of roots and fresh weight as influenced by accession and age of the plants

Treatment combination	*Mean number of roots plant^{-1}*	*Mean fresh weight (g)*
Accession 2 *x* 3 weeks	2.32 ^{a}	0.17 ^{c}
Accession 2 *x* 5 weeks	2.26 ^{a}	0.42 ^{a}
Accession 1 *x* 5 weeks	2.19 ^{a}	0.22 ^{b}
Accession 2 *x* 4 weeks	2.15 ^{ab}	0.24 ^{b}
Accession 1 *x* 4 weeks	1.93 ^{b}	0.13 ^{d}
Accession 1 *x* 3 weeks	1.31^{c}	0.10 ^{e}
CV %	14.95	27.8

Within a column, the means followed by the same letter are not significantly different at p=0.05

Rady (2005) noted that it takes 20 days in MS medium with IBA (3 mg L^{-1}) for shoots to produce the first root. In the present study, the shoots of both the accessions produced more than one root under *ex vitro* conditions within three weeks. The highest fresh weight (0.42 g) was recorded by the five weeks-old plants of accession 2. Furthermore, a higher number of roots was also recorded in accession 2 compared to accession 1. This could be attributed to the vigorous growth habit and a comparatively higher leaf area of accession 2. Mpeck and Atangana (2007) reported that the number of roots per cutting was influenced by leaf area in *Baillonella toxisperma* by interacting with substrate. Therefore, the rooting of stem cuttings can be related to the photosynthetic activity during propagation, which itself is influenced both by the propagator microclimate and leaf area (Mes′en *et al.*, 1997).

Both accessions produced the longest shoots (p=0.0011) in the sand medium (Table 5). A lower shoot length was observed for accession 1 in the soil-based medium. This could be attributed to the lower degree of rooting and resulting in poor shoot growth.

Table 5. Shoot height and fresh weight of the plants as influenced by the treatment combinations of rooting medium and accession

Treatment combination	*Mean shoot height (cm)*	*Mean fresh weight (g)*
Sand *x* Accession 2	4.85 ^{a}	0.36 ^{a}
Sand *x* Accession 1	4.48 ^{a}	0.18 ^{c}
Soil based medium *x* Accession 2	4.05 ^{b}	0.23 ^{b}
Coir dust:sand (3:1) *x* Accession 1	3.90 ^{b}	0.13^{d}
Coir dust:sand (3:1) *x* Accession 2	3.85 ^{b}	0.23 ^{b}
Soil-based medium *x* Accession 1	3.34 ^{c}	0.10 ^{d}
CV %	13.45	27.8

Within a column, the means followed by the same letter are not significantly different at p=0.05.

The highest fresh weight (p=0.0023) was recorded in the accession 2 planted in sand medium. Statistically similar fresh weights were also recorded for the same accession in other media tested ($p>0.05$). The accession 1 exhibited a similar trend in fresh weight though the values were much lower than those of the accession 2. The difference in the fresh weights in accessions (Table 4) could be attributed to the morphological differences of the accessions than the treatment effect. Further, shoot height increased with the age of the plants and the mean shoot heights were significantly different ($p=0.0001$). The mean shoot heights of both varieties were 4.53 cm at five weeks, 4.01 at four weeks and 3.69 cm at three weeks.

Though a proper cost analysis was not performed for this technology, it was evident that a significant cost reduction could be achieved in terms of saving electricity, chemicals, time and labour. The results also demonstrated the efficiency of an ordinary propagator in facilitating rooting and growth of *Gypsophila* microshoots. Using this protocol, about 750 plants m^{-2} can be rooted in less than one month period.

CONCLUSIONS

Results showed that *G. paniculata* can be successfully multiplied *in vitro* through single nodal segments in MS medium supplemented with 1 mg L^{-1} BAP. Rooting of microshoots of *G. paniculata* could be conducted *ex vitro*. This would eliminate the sterile rooting stage thus, simplifying the protocol, reducing time and cost of planting material production. Steam-sterilized coarse sand was superior in terms of plant survival, rooting and growth of the plants.

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