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CONTROL OF VITRIFICATION IN MICROPROPAGATED PLANTLETS OF BABY'S BREATH (*Gypsophila paniculata* L.)

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ABSTRACT

Gypsophila paniculata **.L (Baby's Breath) a cut flower repute in Sri Lanka is conventionally propagated by imported seeds. Although *In-vitro* technique being a useful tool for clonal propagation of *G. paniculata,* vitrification is identified as a propagation barrier while regeneration through *In-vitro*. Modification of culture medium in order to overcome this adverse effect was investigated. Shoot tips (1cm) of *G. paniculata* were aseptically established in culture tubes sealed with cotton plugs, containing MS solid medium supplemented with Kinetin (0-1.5 mg/l), BAP (0-1mg/l) and NAA (0-1mg/l). Shoot formation was obtained within 4 weeks found greater in the medium containing 0.5mg/l Kinetin, 0.5mg/l BAP and 0.2 mg/l NAA. Vitrification was occurred in shoots remained more than 4 weeks in the same medium. The shoots were transferred to shoot proliferation medium (1/2 MS) supplemented with Kinetin (0.5-1.5 mg/l), BAP (0-1mg/l) and Charcoal (0-2g/l) and sealed with cotton plugs and aluminium foil. Highest multiplication rate was observed in the medium containing I mg/l Kinetin and 0.5 mg/l BAP (10.7/explant) sealed with the cotton plugs. However, rate of multiplication was reduced to 7.3/explant and rate of vitrification reduced to 4.8% when charcoal (1 g/l) added to the same medium. The Medium without charcoal and vessels sealed with Aluminium foil increased the vitrification rate (52%). The multiplied shoots were rooted in 1/2 MS medium supplemented with IBA (0.2 mg/l) and charcoal (1mg/l).**

KEY WORDS : *Gypsophila paniculata,* Vitrification

INTRODUCTION

Baby's Breath (*Gypsophila paniculata*) which belongs to the family *Caryophyllaceae* is a cut flower with a high market demand. Since this species has propagation constraints, growers do not get sufficient planting material. Tissue culture techniques are used for mass propagation of *G. paniculata.* Application of *in-vitro* technique has been restricted due to abnormal growth pattern of the plants. Vitrification is identified as the most important barrier in micropropagation of this species. Shoot cultures of many species, may become slow-growing resulting to tightly rolled translucent leaves, a situation, which has been described as vitrification. Occurrence of vitrification is identified as a propagational barrier due to poor survival rate, weakness of regenerated plants, reduction of multiplication rate and slow growth of *in-vitro* cultures (Ahuja, 1993).

Chun *et al., (*1996) reported that in the phase of *in-vitro* proliferation of poplar species, vitrification symptoms appeared more in the cultures of liquid medium. Ahuja (1993) showed that at the *in-vitro* multiplication stage of apple, some of the shoots showed signs of vitrification. This condition could be converted to normal type by reducing macroelements of Murashige and

Skoog medium (1962) to half strength and increasing agar concentration to 1.5%.

Castro *et al.*, (1996) found that as the concentrations of IAA reduced to 0.5 mg/l in the MS medium used for meristem culture of *G. paniculata*, rate of vitrification decreased in regenerated plantlets. It was also shown that magnitude of vitrification in *G.paniculata* was lowered when vessels were sealed with Milli wrap compared to aluminium foil, indicating the role of CO_2 and Ethylene accumulation in the vessels in vitrification (Han-Bong *et. al.*, 1993). It was observed that vitrified plantlets of *G. paniculata* existed the malformed stomata, discontinuous holes in the epidermis of the leaves as compared to the non-vitrified plants (Gribble *et al.*,1996*)*. Han (1991) reported that as the agar concentration increased, fresh weight of the plantlets, shoot number and the rate of vitrification were decreased in the shoots of *G. paniculata*. Shoots cultured at 22 ° C had significantly less vitrification than those cultured at 26-28° C. Since vitrification is identified as a draw back for continuous propagation of *G. paniculata in-vitro*, this study was carried out to perfect the technology for mass propagation of *G. paniculata* with the minimum level of vitrification.

MATERIALS AND METHODS

Axillary buds of *G. paniculata* (cv.Bristol Fairy) were collected from pot grown greenhouse plants, which were treated with fungicide (Benlate 2%) 3 days prior to removal. The material were washed thoroughly under running tap water and surface sterilized with 10% Clorox (1% active chlorine) for 10 minutes followed by washing four times with sterilized distilled water. Shoot tips (1 cm) were aseptically established in culture tubes (25 mm x 150 mm) containing MS (Murashige and Skoog, 1962) medium supplemented with different hormones combination described in table 1.

Table 1. Description of media (mg/l) for culture establishment and proliferation.

Culture establishment				*Proliferation*			
Medium No	*Kinetin*	*BAP*	*NAA*	*Medium No*	*Kinetin*	*BAP*	*Charcoal*
1	0.25	0.1	0	1	0.5	0.5	0
2	0.25	0.2	0.1	2	1.0	0.5	0
3	0.25	0.3	0.2	3	1.5	1.0	0
4	0.5	0.4	0.2	4	0.5	0.5	1
5	0.5	0.5	0.2	5	1.0	0.5	1
6	0.5	0.6	0.3	6	1.5	1.0	1
7	0.75	0.7	0.3	7	0.5	0.5	2
8				8	1.0	0.5	2
				9	1.5	1.0	2

Shoots regenerated from the shoot tip derived explants were transferred singly to the wider culture vessels (200 ml) sealed with cotton plugs and aluminium foil, containing 25 ml of proliferation medium (1/2 MS) supplemented with different hormones and charcoal levels (table 1). Sub culturing was done using the 1/2 MS medium supplemented with the IBA (0-1mg/l) and Charcoal (0-1 g/l) for root induction.

All the cultures were incubated under fluorescent light illuminant of 120μE $m^{-2}s^{-2}$ for a period of 16 hours per day at the temperature of 26±1°C. The experiment for shoot proliferation phase was arranged in completely randomized design with 15 replications. Data were collected on the rate of proliferation and rate of vitrification. Vitrified plants were identified with the symptoms of tightly rolled, translucent and discoloured leaves and stems. Rate of vitrification was calculated as number of vitrified plants appeared out of the total proliferated plants. Data were subjected to statistical analysis as 2 factor design using F test at significant level from 0.1 -0.5%.

RESULTS AND DISCUSSION

Statistical analysis of the data shows that there is a significant effect of the medium for multiplication rate and the sealing material on the rate of vitrification. The significant interaction between medium and sealing materials on vitrification rate was also observed (table 2 and 3). However, there no significant interaction was observed between these two factors on multiplication rate.

Primary culture establishment

Successful primary culture establishment was achieved with the shoot tip explants cultured in the MS medium containing 0.5 mg/l Kinetin 0.5 mg/l BAP and 0.2 mg/l NAA among the different concentrations tested. Maximum number of shoots (3.1/explant) and plant height (4.5 cm) were obtained from the same medium (table 2).

Table 2. Effect of different growth regulators on shoot formation (Number of shoots/explant in 4 weeks of culture)

Medium Number	*Average Number of Shoot/explant*	*Average plant height (cm)*
1	0.87	3.5
2	1.94	3.2
3	2.42	4.1
4	2.73	4.2
5	3.10	4.5
6	2.83	4.6
7	2.74	3.8

Occurrence of vitrification was observed in the shoots remained more than 4 weeks in the same medium. The results suggested that micro shoots derived from the primary explants may be taken within the period of 4 weeks of growth for sub-culturing in order to avoid vitrification.

Rate of multiplication:

Highest multiplication rate (10.78/explant) was observed in the MS medium containing 1 mg/l Kinetin and 0.5mg/l BAP (table 3).

Table 3. Effect of different growth regulators and the sealing materials of the vessels on the multiplication rate (# of shoots/explant) *G. paniculata.*

Medium	*Sealing material*		*Average*
	Cotton plugs	*Aluminium foil*	
1	8.34	6.64	7.49
2	10.78	9.50	10.14
3	10.38	8.24	9.35
4	6.42	5.20	5.85
5	7.24	5.32	6.28
6	6.82	4.84	5.83
7	6.50	5.46	5.98
8	6.74	5.20	5.97
9	6.42	5.12	5.77
Average	7.73	6.16	

LSD (P=0.05) for medium = 2.26; LSD (P=0.05) for sealing material = 1.06
Interaction (medium x sealing material) was not significant; CV (%) = 16.27

Multiplication rate was reduced significantly ($p<0.05$) when charcoal was added to the medium. Significant difference was observed between the cultures sealed with cotton plugs and aluminium foil in respect of multiplication rate (figure 1[1]). There is no significant interaction ($p>0.05$) between media and sealing material on multiplication rate. It suggests that

[1] Figures are provided in a plate at the the end of the paper.

influence of sealing materials behave uniformly in different medium on multiplication rate (table 3)

Rate of vitrification:

There is a significant influence of sealing material and the medium on the rate of vitrification (table 4). Significant increase in vitrification was observed when cultures do not contain charcoal and sealed with Aluminium foil (figure 1). It was reported that accumulation of ethylene and CO_2 would result in vitrification of *G. paniculata* (Han-Bong the *et al.*, 1993). As reported by Mensulai (1993), accumulation of gasses such as ethylene and CO_2 produced by *in-vitro* cultures could be released through gas exchangeable sealing material and absorbed by charcoal added to the medium. The low vitrification rate that appeared in the media which contained charcoal in the experiment may be due to absorption of gasses (ethylene) by charcoal and releasing these gasses to atmosphere by sealing of the cultures with gas exchangeable material (cotton plugs).

Table 4. Effect of different growth regulators and the sealing materials of the vessels on the vitrification rate (percentage) of *G. paniculata*.

Medium	*Sealing material*		*Average*
	Cotton plugs	*Aluminium foil*	
1	9.1	42.4	25.75
2	10.6	52.6	31.6
3	11.3	39.0	25.1
4	7.3	16.3	11.8
5	4.5	18.2	11.35
6	5.4	16.2	10.8
7	5.7	16.6	11.15
8	6.3	15.2	10.75
9	6.0	14.6	10.3
Average	7.35	25.6	

LSD (P=0.05) for medium = 2.32; LSD (P=0.05) for sealing material = 1.09
Interaction between media and sealing materials was significant; CV (%) = 10.74

The interaction between medium and sealing material was significant at 5% level with respect to rate of vitrification. This shows that sealing material do not have the same influence over different media due to their differences in gas accumulation. This could be due to charcoal treatment in some of the media.

Cuttings taken from the *in-vitro* proliferated shoots successfully rooted in 1/2 MS medium supplemented with 0.2mg/l IBA and 1g/l Charcoal within the period of 4 weeks. The results of this experiment suggest that presence of

charcoal in the medium and sealing with cotton plugs could reduce the vitrification of *G. paniculata in-vitro.*

CONCLUSION

The minimum level of vitrification (4.5%) and highest rate of multiplication (7.3/explant) were obtained in the cultures containing _ MS Medium supplemented with 1mg/l Kinetin 0.5 mg/l BAP and 1g/l Charcoal and the cultures vessels sealed with cotton plugs. Use of this modification could be recommended for the *in-vitro* multiplication phase of *Gypsophila paniculata.*

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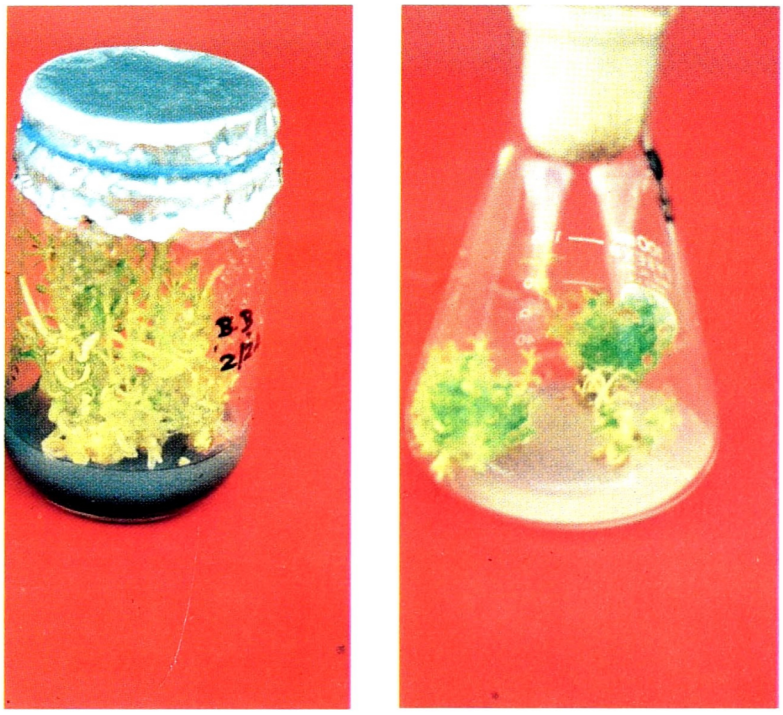

Figure 1. Vitrification of *in-vitro* plantlets of *G. paniculata* in the vessels sealed with aluminium foil and cotton plugs.

PLATE I