IN VITRO PROPAGATION OF *GYPSOPHILA PANICULATA* L. THROUGH PLANT TISSUE CULTURE TECHNIQUES

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ABSTRACT: Apical meristem as explant was used to micropropagate the economically important cut flower plant, *Gypsophila paniculata* L. by using *in vitro* techniques. Different media were used for plant propagation, and multiple shoots were obtained on MS medium fortified with different concentrations of BAP along with various combinations of NAA and Kin. After removing the leaves, apical meristems were washed with tap water and household detergent and also dipped in commercial sodium hypochlorite (bleach) for sterilization. Then these sterilized plants are inoculated on MS medium supplemented with different plant growth regulators like BAP (at concentrations of 0.25, 0.5, 1.0, 1.5 & 2.0 mg/l), for multiplication, different concentrations of BAP and NAA, combinations of BAP and Kin (mg/l), BAP1 + Kin (mg/l) combinations of BAP1, Kin and NAA (mg/l) were used, for rooting different concentrations of NAA (0.1, 0.2, 0.3, 0.4 & 0.5 mg/l) were used. The optimum temperature for plantlets formation in culture room was maintained at 22±2˚C. The cultures were incubated at 16 hours of light period with light intensity of 2000-3000 lux. All media were based on the MS basic salts, (macro & micro), iron EDTA, vitamins, sucrose 30g/l and 0.1g of myoinasitol. The pH of media was adjusted at 5.57, and the cultures containing 1.5g/l of phytagel before autoclaving at 121 C° 15lb/inch^2 for 20 minutes. Among all the media tested the highest number of shoot emergence, multiple shoot formation, and maximum shoot length was obtained in medium supplemented with BAP 1mg/l after 4 days of inoculation. Which shows the maximum shoot length of 6.53 (cm) with 86 percentage response observed in same medium after 21 days. For root initiation the best medium was NAA 0.5 mg/l which showed 85 % of root initiation with length of 2.66 (cm) after 11 days of inoculation. A completely randomized design was used for the experiment with three replicates. The data was analysed by COSTAT V.63: statistical software (Cohort software, Berkely, California) and Duncan' New Multiple Range.

Key words: *Gypsophila paniculata* L.; Murashige & Skoog's; Shoot initiation; Root initiation.

INTRODUCTION

Gypsophila paniculata L. is a perennial plant belonging to the family Caryophyllaceae. The family Caryophyllaceae is native to Europe, Asia and North Africa. Many species are found on calcium rich soils, including gypsum, which is depicted in the name of the genus, "*gypsophila."* Some species are also sometimes called "baby's breath" or simply, "Gyp", among the floral industry. Its botanical name means "lover of chalk", which is accurate in describing the type of soil in which this plant grows (Walker, 1994).

Gypsophilas are often grown as ornamental plants in gardens, they are grown both as garden plants and also valuable as a cut flower in floristry to add as a filler to flower bouquets (Rehman, 2002). It is an herb which can reach 1.5 m in height, the most important part being underground. The roots are ramified with long, thick branches. The upper shoot is erect. The stems are slender, erect to spreading, swollen at nodes with leaves reduced to an enlarged and flattened petiole. The leaves are small, opposite, lanceolate, often falcate (sickle shaped) with bluish green colour. Inflorescence is profusely branched panicle. It has white or pink flowers, which are composed of a short calyx split almost to the bottom (Hanafya and Lobna, 2007; Ditomaso, 2007).

Gypsophila is worldwide in use in floral industry. The commercial Merk Saponins, which has been widely used as a standard for the haemolytic tests, was obtained from *Gypsophila paniculata*, G. *arrostii* and several other species of Gypsophila. In folklore medicines it was used as remedy for the coughs, colds and the ailments of the upper respiratory tract. Saponins of *G. paniculata* are also in use as a source of variety of products like cleaning chemicals, film emulsions and as ingredient of fire extinguishers, the saponins are being researched for their usefulness in treating leukaemia (Rehman, 2002).

G. *paniculata* plants are sterile and do not produce seeds, therefore, breeding programs are severely restricted (Shillo and Abraham, 1982). The major issue is phytoplasma disease, which has been identified in

Gypsophila (Gera *et al*., 2005). Another main problem is low rooting frequency of vegetatively propagated cuttings which hinders propagation so, the application of tissue culture techniques for Gypsophila is very vital to overcome problems for breeding and propagation. Now, commercial propagation of ornamental plants by tissue culture is being a new promising industry in horticultural markets (Han *et al*.1991, Zamorano-Mendoza and Mejia-Munoz, 1994, Song *et al*. 1996, Lee and Bae, 1999). The goal of this work was to improve *in vitro* technology to propagate *G. paniculata* through shoot tips.

MATERIALS AND METHODS

The plants used as explants were taken from the Lawrence Garden, Lahore Pakistan. First of all shoot tips as explants were taken from the plants. After removing the leaves, apical meristems of 2mm- 4mm were used and whole meristem 6mm was also used and washed with tap water for 5-6 times to remove the impurities without damaging the young and delicate tissues. The Gypsophila explant was then washed with household detergent and rinsed with distilled water for 3 times then it was also dipped in commercial sodium hypochlorite (bleach) (15-22 %) for 15-20 min. After this, the explants were rinsed with autoclaved double distilled water five to six times, so as to reduce the toxic effect of Sodium hypochlorite (bleach) to remove contaminants. (The detergent acts as a wetting agent and allows the entire surface of the leaf exposed to the sodium hypochlorite. This process would remove the surface contaminants such as; exterior bacteria, fungi, spores, mites, or small insects.) Then these sterilized plants are inoculated on MS (Murashige and Skoog, 1962) medium supplemented with different plant growth regulators like 6-Benzylaminopiurine (at concentrations of 0.25, 0.5, 1.0, 1.5 & 2.0 mg/l), for multiplication, different concentrations of BAP and NAA, combinations of BAP and Kin (mg/l), BAP1 mg/l + Kin (mg/l) combinations of BAP 1.0 mg/l, with different concentrations of Kin and NAA (mg/l) were used, for rooting different concentrations of NAA (0.1, 0.2, 0.3, 0.4 & 0.5 mg/l) were used. The optimum temperature for plantlets formation in culture room was maintained at 22±2˚C. The cultures were incubated at 16 hours of light period with light intensity of 2000-3000 lux. Dark period of 8 hours is required alternatively with above describe photoperiod. All media were based on the MS basic salts, (macro & micro), iron EDTA vitamins, sucrose 30 g/l and 0.1g of myoinasitol. The pH of media was adjusted at 5.57, and the cultures containing 1.5g/l of phytagel before autoclaving at 121 C° 15lb/inch^2 for 20 minutes.

A completely randomized design with 3 replicates was used for the experiment. The data for each parameter were subjected to COSTAT V.63: statistical software (Cohort software, Berkely, California). Mean values were compared by applying Duncan' New Multiple Range Test at 5% level.

RESULTS AND DISCUSSION

The low rooting frequency and sterile nature of the Gypsophila plant making it essential to grow it by tissue culturing. By applying all the *in vitro* techniques for the efficient growth the 82% of explants remaining aseptic and showing new growth and development, which shows its successful culturing and establishment in MS medium containing different concentrations of auxins and cytokinins. The Gypsophila cultured on MS medium with different concentrations and combinations of cytokinins and auxins are shown in the Table 1 and the study of the cultures showed that the proliferation capacity of cultured shoot-tips depends on the concentration of the BAP used. While using different concentrations of BAP (0.25, 0.5, 1.0, 1.5 & 2.0 mg/l) the best response was seen in BAP 1.0 mg/l, with 3.66±0.47 time of shoot regeneration and proliferation with maximum shoot length of 6.53±0.36 (Fig. 1a). The effect of different levels or concentrations of BAP with different level of NAA on adventitious shoot proliferation by shoot tip culture of *G. paniculata* was shown in Table -1.By using different combinations and concentration the best combination was optimized. It could be observed that the inclusion of 1.0 mg/l of BAP and 0.2 mg/l of NAA in the culture medium led to the highest shoot length (5.21±0.21) as compared to that recorded with other treatments. Ayeh *et al*., (2009) reported that *in vitro* media contain MS media supplemented with NAA 0.05 mg/l and BAP 1 gave the good response percentage. Hanafy and Lobna (2007) described the shoot tips of *G. paniculata* were cultured on MS-medium contained 0.5 mg/l each of NAA and BAP for establishment of shoot cultures which gives the best result.

In general, high or low concentration of BAP with NAA resulted in lower values for number of shoot and shoot length. The synergistic effects of Kin in combination with BAP were observed and using different concentrations of BAP and Kinetin (0.5, 1.0, 1.5, 2.0 of both and 2.0 BAP and Kin 1.0 mg/l). The best response was observed in 1.0 mg/l of each BAP and Kin with highest percentage of shoot length with 5.32^{ab}±0.42 time .While repeating the experiments with constant concentration of BAP 1 and varying the concentrations of Kin (0.05, 0.5, 1.0, 1.5, 2.0 mg/l) the highest response was again observed in BAP 1.0 & Kin 0.05 mg/l with respect to the percentage of shoot initiation, while the maximum height was obtained in medium containing 1-1 ratio of BAP and Kin (Table-2 and Fig. 1 c-d). Quoirin *et al.*, (2008) reported the usage of Kin for the Gypsophila plant.

With different combinations of BAP1.0 mg/l, NAA and Kinetin the highest response was observed in

Table-1. Effect of different concentrations of BAP and NAA on *in vitro* initiation of shoots of G. paniculata.

Sr. No	Ms media + Growth regulators mg/l	Time for Shoot Initiation (days)	No. of shoots Per explants	Percentage of Shoot initiation (%)	Shoot length (cm)
BAP					
1	0.25	$5.33^{a}\pm0.39$	$1.42^{d}\pm0.36$	70	$3.76^{c}\pm0.55$
	0.5	$4.33^{ab}\pm0.45$	$2.0^{c}\pm0.21$	73	$3.73^{c}\pm0.30$
	1.0	$3.66^{b}\pm0.47$	$5.2^{a}\pm0.08$	86	$6.53^{a}\pm0.36$
	1.5	$4.33^{ab}\pm0.38$	$4.8^{ab}\pm0.13$	76	$5.60^{b}\pm0.21$
	2.0	$4.00^{ab}\pm0.81$	$3.14^{b}\pm0.17$	73	$4.40^{c}\pm0.32$
BAP + NAA					
2	0.5+0.05	$6.33^{a}\pm0.47$	$2.0^{d}\pm0.48$	73	$3.73^{c}\pm0.16$
	0.5+0.1	$5.23^{ab}\pm0.39$	$2.21^{c}\pm0.31$	86	$4.13^{b}\pm0.04$
	1.0+0.2	$5.35^{ab}\pm0.45$	$5.1^{a}\pm0.51$	76	$5.21^{a}\pm0.21$
	1.0+1.0	$5.13^{ab}\pm0.37$	$5.0^{ab}\pm0.13$	83	$5.16^{a}\pm0.30$
	2.0+0.5	$4.66^{b}\pm0.35$	$3.42^{b}\pm0.17$	66	$3.83^{c}\pm0.12$

Table-2. Effect of different concentrations of BAP and Kin on *in vitro* initiation and multiplication of shoots of *G. paniculata*

Sr. No	Ms media + Growth regulators mg/l	Time for Shoot Initiation (days	No. of shoots Per explants	Percentage of Shoot initiation (%)	Shoot length (cm)
BAP + Kin					
3	0.5+0.5	$4.66^{b}\pm0.37$	$1.8^{c}\pm0.39$	73	$4.00^{d}\pm0.08$
	1.0+1.0	$5.32^{ab}\pm0.42$	$5.2^{a}\pm0.19$	86	$5.23^{a}\pm0.05$
	1.5+1.5	$4.31^{b}\pm0.49$	$5.0^{a}\pm0.20$	80	$4.96^{b}\pm0.03$
	2.0+2.0	$5.33^{ab}\pm0.38$	$4.9^{b}\pm0.13$	72	$4.73^{c}\pm0.06$
	2.0+1.0	$6.33^{a}\pm0.47$	$2.62^{ab}\pm0.17$	66	$3.93^{d}\pm0.04$

No. of test tubes cultured = 10 ,Each value is mean of three replicate with standard error (mean ± S. E) a, b, c. Mean followed by different letters in the same column differ Significantly at P = 0.05 according to Duncan's new multiple range test

Table-3. Effect of different concentrations of BAP1, Kin and NAA on *in vitro* initiation and multiplication of shoots of *G. paniculata*

Sr. No	Ms media + Growth regulators mg/l	Time for Shoot Initiation (days	No. of shoots Per explants	Percentage of Shoot initiation (%)	Shoot length (cm)
BAP +Kin					
4	1.0+0.05	$5.66^{a}\pm0.42$	$4.7^{b}\pm0.28$	86	$5.30^{b}\pm0.08$
	1.0+0.5	$4.56^{a}\pm0.47$	$5.2^{a}\pm0.28$	83	$5.43^{b}\pm0.06$
	1.0+1.0	$4.61^{a}\pm0.47$	$5.0^{a}\pm0.1.5$	83	$6.10^{a}\pm0.05$
	1.0+1.5	$4.46^{a}\pm0.37$	$3.2^{c}\pm0.19$	63	$4.83^{c}\pm0.08$
	1.0+2.0	$5.33^{a}\pm0.41$	$2.50^{d}\pm0.16$	66	$3.86^{d}\pm0.07$
BAP +Kin + NAA					
5	1.0+0.5+0.05	$6.66^{a}\pm0.47$	$4.5^{ab}\pm0.41$	83	$5.76^{a}\pm0.14$
	1.0+1.0+0.5	$6.00^{a}\pm0.81$	$4.9^{b}\pm0.30$	83	$5.73^{a}\pm0.11$
	1.0+1.5+1.0	$8.00^{a}\pm0.81$	$5.0^{a}\pm0.23$	73	$4.83^{b}\pm0.04$
	1.0+1.0+1.5	$5.66^{a}\pm0.71$	$2.9^{c}\pm0.28$	63	$4.50^{c}\pm0.08$
	1.0+1.5+1.5	$6.33^{a}\pm0.35$	$2.10^{d}\pm0.17$	63	$3.93^{d}\pm0.07$

No. of test tubes cultured = 10 ,Each value is mean of three replicate with standard error (mean ± S. E) a, b, c. Mean followed by different letters in the same column differ Significantly at P = 0.05 according to Duncan's new multiple range test

Table-4. Effect of different concentrations of NAA on *in vitro* initiation of root *G. paniculata*

Sr. No	Ms media + Growth regulators mg/l	Time for root Initiation (days)	No. of roots Per explants	Percentage of Root initiation (%)	Root length (cm)
NAA					
6	0.1	14.33^{a}±.047	0.0^{d}±0.00	63	1.33^{c}±0.07
	0.2	15.33^{a}±0.81	0.0^{d}±0.00	68	1.46^{c}±0.047
	0.3	13.33^{b}±0..43	1.0^{c}±0.13	75	1.73^{b}±0.08
	0.4	13.0^{b}±0.82	1.5^{b}±0.37	77	1.83^{b}±0.08
	0.5	11.3^{c}±0.45	1.6^{a}±0.20	85	2.66^{a}±0.12

No. of test tubes cultured = 10 ,Each value is mean of three replicate with standard error (mean ± S. E) a, b, c. Mean followed by different letters in the same column differ Significantly at P = 0.05 according to Duncan's new multiple range test.

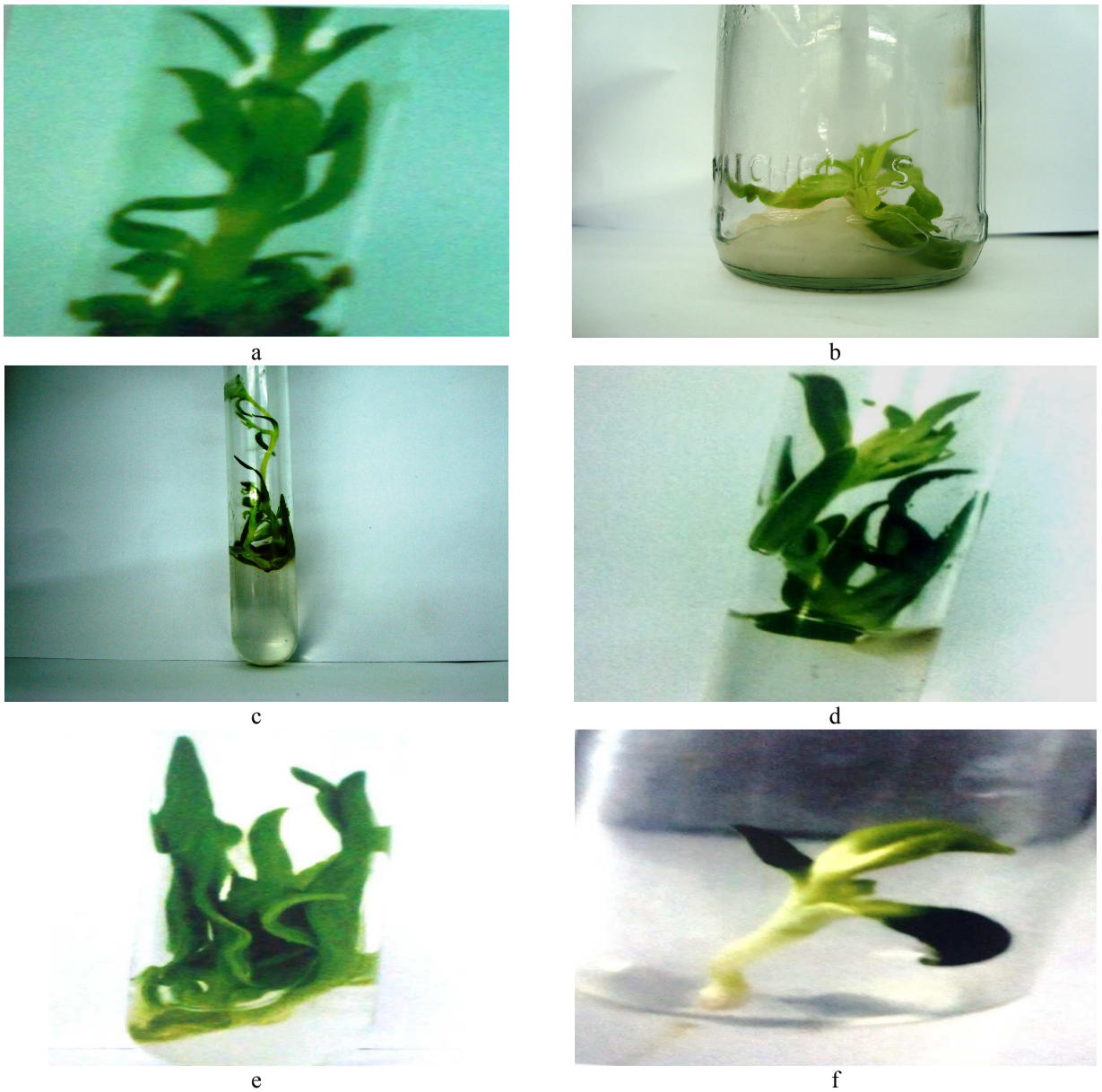

Fig-1: a) Emergence of shoot in MS+BAP1 mg/l in *G. paniculata* b) Initiation of multiple shoots in MS+BAP+NAA c) Multiplication in MS+BAP + Kin mg/l d) Multiple shoot formation in MS + BAP1+ Kin mg/l e) Mass multiplication of micro shoots in MS+BAP+NAA + Kin f) Root formation in MS+NAA.

BAP1.0 NAA 0.5 mg/l and Kinetin 0.05 and also with BAP 1.0 NAA 1.0 and Kinetin 0.5 mg/l, which resulted in highest multiplication rate, and showing up to 83% of shoot initiation, growth and development (Table-3 and Fig. 1 d-e). Ozcelik and Kepenek (1996), Song *et al.,* (1996) also reported that BAP, Kin, NAA and IBA in combination of each other prove the best media for micropropagation or *in vitro* growth of Gypsophila plant. For rooting the plantlets are shifted in medium containing different concentrations of NAA alone and gave 85% age of response in medium containing 0.5mg/l of NAA in respect to the other medium containing 0.1,0.2,0.3and 0.4 mg/l of NAA (Table-4 and Fig. 1 f). Ayeh *et al*, (2009), Zuker *et al.,* (1997) repoted the NAA as the best medium for root development of Gypsophila plant.

For acclimatization process the plantlets of Gypsophila were first transferred to green house with the highest humidity and with all other necessary environmental condition in the disinfected, highly drained soil, preferably calcareous soil with the pH range between 6.5- 7.5. The best soil to grow gypsophila is sand: soil: peat with 1:1:1 ratio After 30-40 days the plants are shifted to complete sunlight from the green house ,and after another two weeks the plants are successfully transformed to fields. As temperature and day length decrease, gypsophila tends to rosette. The results obtained *via* plant tissue culturing in present goal could be highly promising to increase the regeneration capacity, high multiplication rate and yield improvement of *Gypsophila paniculata.*

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