IN VITRO MICROPROPAGATION OF *GYPSOPHILA MURALIS* L. FROM COTYLEDON EXPLANTS

MICROPROPAGAREA *IN VITRO* DIN EXPLANTE DE COTILEDON LA *GYPSOPHILA MURALIS* L.

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Abstract. *This study examined the effects of different exogenous auxins and cytokinins at 1.0-5.0 mg·l-1 concentration on morphogenetic response of cotiledonary nodes of Gypsophila muralis seedlings. Their sensitivity to the auxins varied and showed concentration-dependent behavior, and the response differed between two used auxines. The number of responding explants was higher on the media supplied with 2,4D but on the media suplimented with IAA produced fast growing yellow and green compact callus, which showed beneficial effect on the cell growth of morphogenic cultures. Increased cytokinins led to differentiation of shorter shoots. The culture media fortified with BAP in combination with IAA produced yellow-green compact morphogenic callus witch develop multiple shoots*

Key words: *Gypsophila muralis*, in vitro, auxins, cytokinins

Rezumat. *Prezentul studiu a avut ca scop evaluarea efectului unor concentraţii diferite de auxine şi citochinine exogene asupra reacţiei morfogenetice ale explantelor de noduri cotiledonare obţinute din plantule de Gypsophila muralis. Reacţia obţinută denotă o dependentă atât în funcţie de concentraţia cât şi de tipul regulatorilor de creştere adaugaţi în mediul de cultură. Astfel, proliferarea cea mai intensă a calusului s-a obţinut pe mediile suplimentate cu 2,4D, în schimb adăugarea AIA în mediul de cultură a condus la obţinerea unui calus verde, compact, cu potenţial organogen ridicat. Creşterea conţinutului de citochinine induce diferenţierea lăstarilor, cele mai bune rezultate pentru acest proces fiind obţinute prin utilizarea combinaţiei de BAP şi AIA.*

Cuvinte cheie: *Gypsophila muralis*, in vitro, auxine, citochinine

INTRODUCTION

Plant tissue culture can be applied to the rapid propagation and ex situ conservation of rare, endemic, and endangered plant as explained by several authorities (Purohit et al, 1994; Krishnan et al., 1995; Sudha and Seeni, 1996). Moreover, biotechnology offers tools which are capable of surpassing some limitations found by traditional plant breeding methods, by developing new material, through tissue culture techniques. However, for the use of these possibilities it is necessary to develop of *in vitro* plant regeneration protocols, allowing the recovery of improved material for further propagation.

Gypsophila muralis is a plant that for a long time has been ignored by gardeners, but very suitable for preparing impressive baskets and patio containers

or for bedding. Forming neat and tidy compact bushes of a delicate light- green foliage, this plant is covered all summer by numerous soft pink flowers.

Besides its ornamental characteristics, this specie has a great adaptive potential to a variety of environmental conditions. These features offer a good reason for using this specie to enlarge the ornamental plant pool through in vitro propagation.

The conventional method of propagation of *Gypsophila muralis* is through seed and is not attractive approach for producing a large number of elite plants within short period. Further its starting material for research is met solely from the wild natural population this leads to their gradual depletion, and as a result of which many plants is now listed as a rare species. Therefore, the measures to develop micropropagation protocols for elite stocks of rare and economical important species are urgently needed. Some studies have reported *in vitro* plant regeneration and micropropagation of *Gypsophila paniculata* (Han *et al*.1991a, Zamorano-Mendoza and Mejia-Munoz, 1994, Song *et al*. 1996, Lee and Bae, 1999 etc.), but no information exists on in vitro cultivation for *Gypsophila muralis* specie.

This work was therefore aimed at investigating the effects of supplementing the basal medium with different growth regulators on *Gypsophila muralis* cotyledonary nodes explants cultured *in vitro*.

MATERIAL AND METHODS

The basal MS media supplemented with different concentrations of plant growth regulators in various combinations have been given in Tables 1. All the initial culture media were formulated with 30.0 g L-1 sucrose and 7.5 g L-1 agar and autoclaved at 121^0C under 1.1 kg cm-2 for 20 min after adjusting the pH to 5.6 ± 0.1 with 1 N NaOH. Readymade MS medium were procured from Sigma, US.

Seeds of *Gypsophila muralis* collected from the Buzău area in 2009, were thoroughly washed under running tap water for five minutes before surface sterilizing with 70 % (v/v) ethanol for 1 min followed by a treatment of 0.1 % (w/v) HgCl2 for 3 min and finally 4-5 times rinsing with sterilized water. Surface sterilized seed were inoculated in culture tubes containing agar gelled water (7.5 g L-1 agar) under diffused luminance of 16µ mol m-2 s-1 provided with PAR for seven days. Cotyledonary node explants were obtained from 5- or 7-day-old seedlings and were plated in glass tubes containing 40 ml of MS (Murashige and Skoog, 1962) basal medium and incubated under complete darkness at 25 ± 2ºC for 1 week. Later the cultured tubes were subjected to 16 h photoperiod regime of 30 µmol m-2 s-1 luminance provided with white PAR lamps.

Table 1

MS media used in the experiments

Growth regulators	MS1	Ms2	Ms3	Ms4	Ms5	Ms6	MS7	MS8	M S9
2,4D	1	1	1				1		
AIA			1	2	5	1	1	5	1
K	1						1	2	2
BAP		1	1	1	2	2			

The variables studied in the three experiments were percent of responsive explants and morphogenetic response of plants placed on different media formula.

RESULTS AND DISCUSSIONS

All the cultured explants enlarged during initial 7-8 days and no callus proliferation was observed. During this period explants responded in a similar manner mostly independent from culture media and conditions. During second week of culture, morphogenetic response of explants was different and depends of growth regulator balance of culture media. Where as from some explants profuse callus growth started from the cut ends. The calli from various explants generated on different media combinations varied in texture and color. Discrete phenotypes of proliferated calli were observed viz. wet, rough, fragile, dense and glossy in texture and white, dark/ light green and yellow in color.

Visual selection and sub-cultures of these pheno-variants produced cultures where plantlet regenerated repeatedly and competently (fig.1 A-I). In *Gypsophila*, although, plants from tissue cultures have been regenerated on an array of basal medium such as MS (Rady, 2005) and MH3 medium (Gevrenova et al., 2010**)**, in our experiments, MS basal medium was used throughout the experiments, as it was found responsive to *Gypsophila muralis* specie.

During course of present investigations two auxins (IAA and 2,4-D) and two cytokinins (BAP and Kinetin) were used singly as well as in a number of combinations and concentrations (table 1) for culture establishment. Results clearly indicated varying response of growth regulators on explants morphogenetic reaction. Higher callus initiation was observed on culture media fortified with an cytokinins or at the minimum on 1/1 auxine cytochinines ratio. At lower levels both the auxins 2,4-D or IAA have been found to initiate callus proliferation, however, such calli failed to produce normal plants. At higher levels of auxins (2 0 mg L-1) callus turned blackish with retarded growth and cell mortality was observed. At 5 mg L-1 of IAA no callus formation is observed. The meristematic apex is growing, and develops shoots.

Maximum callus initiation was observed on culture medium containing 2,4D. Culture media containing IAA produced fast growing yellow and green compact callus, which showed beneficial effect on the cell growth of morphogenic cultures.

Callus produced in media supplemented with kinetin resulted in the formation of hairy roots with lower frequency of shoot formation during the advanced phase of cultures. Among two levels of cytokinins tested, lowest level (1 mg L-1) stimulated growth of morphogenic tissues

With a higher levels of BAP multiple shoots proliferated from meristematic zones without intervening callus phase. Rate of recurrence of callus was higher when an auxin was supplemented to the cytokinin in culture medium. However in both the cases, frequency of morphogenic calli was found to be low.

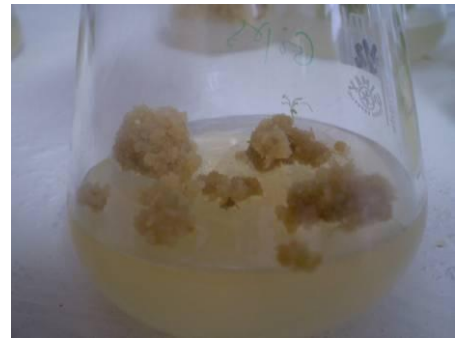
Medium containing 2,4-D with BAP or K did not initiated much of the morphogenic cultures and they fail to regenerate in the regeneration medium in spite of being large and pale yellow in colour. On the other hand, the culture media fortified with BAP in combination with IAA produced yellow-green compact morphogenic callus.

Table 2

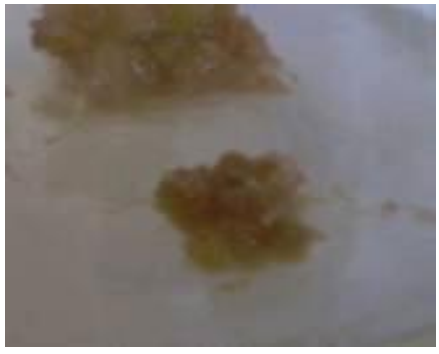
Response of cotyledonary nodes explants of *G. muralis* in MS with BAP, KN, IAA and 2,4 D

Variant	Response of cotiledonary nodes explants (after 35 days)	%
MS1	White friable callus, non-regenerative	98
MS2	Green friable callus developed from the cut end of the explant	95
MS3	Green compact callus	95
MS4	Dark compact callus, non-regenerative	86
MS5	A few rows of cells emerged in the margin of explant and multiple shoots developed from meristematic apex	89
MS6	Shoots proliferated from meristematic zones without intervening callus phase	91
MS7	Yellowish green friable callus	99
MS8	Green multiple shoots developed on green friable callus	97
MS9	Green granular callus	97

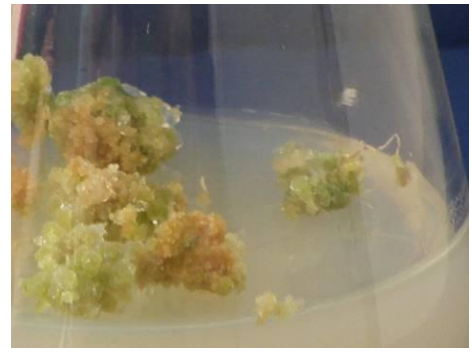
a)



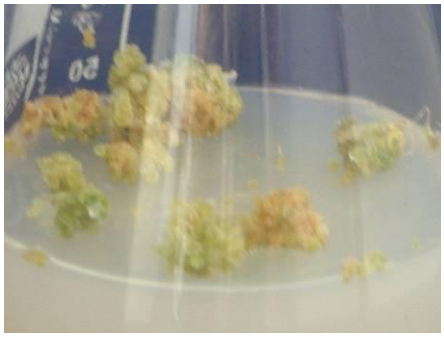
b)



c)

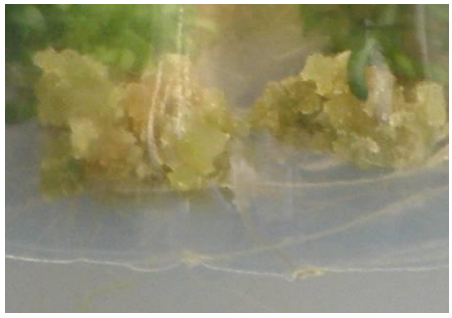


d)



e)

f)



g)

h)

i)

Fig. 1 (a-i). Morphogenetic response of cotiledonary nodes of *Gypsophila muralis* on MS media with different growth regulators: a) white friable callus, non-regenerative on MS1 variant; **b)** non-regenerative, Dark compact callus on MS4 variant; **c)** green granular callus on MS9 variant, **d)** green compact callus on MS3 variant; **e)** yellowish friable callus on MS7 variant; **f)** a few rows of cells emerged in the margin of explant and multiple shoots developed from meristematic apex on MS5 variant; **g)** green friable callus developed from the cut end of the explant on MS2 variant**; h)** green multiple shoots developed on green friable callus on MS8 variant; i) shoots proliferated from meristematic zones without intervening callus phase on MS6 variant,

CONCLUSIONS

1. Higher callus initiation was observed on culture media fortified with an cytokinins or at the minimum on 1/1 auxine cytochinines ratio.

2. At lower levels both the auxins 2,4-D or IAA have been found to initiate callus proliferation, however, such calli failed to produce normal plants but at higher levels of auxins cell mortality was observed.

3. Callus produced in media supplemented with kinetin resulted in the formation of hairy roots with lower frequency of shoot formation during the advanced phase of cultures.

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