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TISSUE CULTURE STUDIES FOR MICROPROPAGATION OF CARNATION (*DIANTHUS CARYOPHYLLUS L.*)

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Abstract

Rapid propagation of carnation (*Dianthus caryophyllus* L) was achieved *in vitro*. Multiple shoots were induced from nodal explants on MS medium supplemented with 1.0, 2.0 or 3.0 mg/l of BAP. For further proliferation the multiple shoots were transferred to MS medium containing different concentrations of BAP and GA_3 alone or in combination. Maximum number of plantlets per flask were produced on medium enriched with 5.0 mg/l BAP. Rooting was stimulated by subculturing the proliferated shoots on 1/2 MS, 3% sucrose and 1% agar. Rooted plantlets were successfully raised in pots filled with soil.

Introduction

Carnation (*Dianthus caryophyllus* L.) also called Grenaid or clove pink are the hardy perennial garden and hot-house flowers of the pink family Caryophyllaceae. They are found in all parts of the world but are most abundant in N.Europe and W.Asia. The plants have not as yet been propagated through adventitious shoots and detached leaves *in vivo*, whereas *in vitro* propagation techniques have been developed (Murashige, 1974, 1978). Most of the tissue culture study on carnation have been directed mainly towards the micropropagation and the production of virus-free plants using meristem as stem tip culture (Stone, 1963). Takayama & Misawa (1982) used a shake culture method for rapid and mass propagation of lilies, begonias, carnations, chrysanthemum etc. Mii & Cheng (1982) obtained callus and observed rooting from mesophyll protoplast of carnation. The present report describes the results of the experiments conducted to induce multiple shoots on stem nodal explants preferably from the apical region of carnation plant and its transfer in soil.

Materials and Methods

Stem nodal explant of *Dianthus caryophllus* L., after washing in tap water to remove dust particles was surface sterilized in 0.5% mercuric chloride for 3 minutes followed by washing in sterilized distilled water. Basal nutrient medium of Murashige & Skoog (1962) and different doses and auxins and cxytokinins along with 3% sucrose and 1% Difco Bacto agar were used. The pH of the medium was adjusted at 5.7 before autoclaving at 15 p.s.i for 15 minutes. Explant were cultured in an upright position and incubated in 16 h daily photoperiod (light intensity 2000 lux at 25 $\pm$ 1°C and 65% R.H. For obtaining multiple shoots, stem nodal explants were excised and cultured on MS medium incorporated with different growth regulators. Two different media were prepared, one for the induction of shoots and the other for their proliferation.

Table 1. Effect of growth hormones on shoot induction in *Dianthus caryophyllus* nodal segments

No. of explants cultured	Hormone concentration (mg/l)	% of explant responded	Culture period (weeks)	Response
20	1.0 BAP	70	1	Multiple shoots at one point and traces of callus
20	2.0 BAP	100	1	Rossete of multiple shoots at one point No callus formed
20	3.0 BAP	89	1	No callus formed
20	5.0 kinetin	20	4	No callus formed
20	0.5 AS + 1.0 BAP	30	4	Very little shoots formed
20	1.0 BAP + 0.1 IAA	40	3	Very little shoots formed
20	0.5 BAP + 0.01 IAA	30	4	Very little shoots formed

Results and Discussion

i) ***Shoot Inducation***: Cytokinin as BAP when used singly @ 1.0-3.0 mg/l induced large number of axillary shoots, with 2.0 mg/l as the optimal concentration. BAP when used in combination with As or IAA did not give satisfactory results. Very scarce shoots were formed when IAA @ 0.01 mg/l was used in combination with 0.5 and 0.1 mg/l BAP. Similarly when 5.0 mg/l As was used with 1.0 mg/l BAP very little shoots were formed (Table 1). These results are in contrast with Roest & Bokelman (1981) who used 1.0 mg/l BAP and 0.01 mg/l IAA for multiplication of carnation.

Kinetin used @ 5.0 mg/l could not induce any shoot formation, however, some swellings in the centre of explant were observed after 4 weeks of culture. Siddique et al., (1993) found that shoot initiation in carnations started at 2.0 mg/l of Kinetin, and maximum number of explants started producing shoots at 5.0 mg/l kn. Schanapp & Preece (1986) also found shoot induction and multiplication at 5.0 mg/l kn.

ii) ***Shoot Multiplication***: BAP @ 5.0 mg/l was found to be the most effective cytokinin for stimulating axillary shoot proliferation (Table 2, Fig.2). When GA_3 @ 0.5-1 mg/l was used alone or with BAP @ 4.0 mg/l, a fairly good amount of multiple shoots were formed but the shoots tended to elongate rather than to multiply. This suggests that GA_3 could be used to induce elongation in *in vitro* raised shoots so as to obtain a reasonable size for root induction.

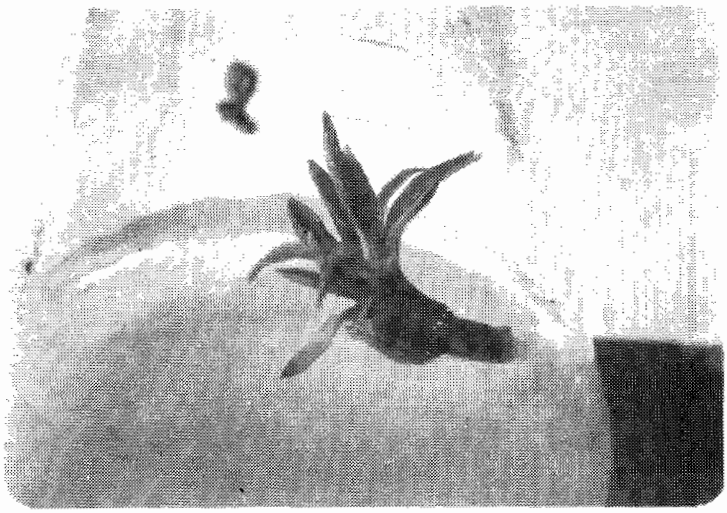

Fig.1. Multiple shoots formed after 1 week of culturing on MS medium supplemented with 2.0 mg/l of BAP.

iii) *Rooting of the Young Shoots*: Plantlets 1.0 cm long were excised and transferred to medium supplemented with either 0.1 mg/l NAA or $1/_2$ MS. Root growth was much faster which started to emerge after 9 days but the roots were small is size and fragile.

Maximum root formation occurred when MS was reduced to half strength (Fig.3) indicating that a higher concentration of nutrients inhibited the formation of roots. In

Fig.2. Subculture of excised shoot from a medium containing 2 mg/l of BAP on BM provided with 5.0 mg/l of BAP after 4 weeks of inoculation.

Fig.3. Root formation in plantlets obtained from MS medium supplemented with 5 mg/l of BAP on transfer to MB supplied with 1/2 MS medium.

Table 2. Effects of different concentrations of BAP and GA_3 on shoot proliferation in *Dianthus caryophyllus* shoots taken from induction medium.

Previous Medium Hormone concentration (mg/l)	Present Medium Hormone concentration (mg/l)	Multiple shoot formation	Duration (weeks)	Results
MS + 2.0 BAP	MS+4.0 BAP+0.5 GA_3	Good	4	Good amount of shoots formed
MS + 2.0 BAP	MS+4.0 BAP+1.0 GA_3	Good	4	"
MS + 2.0 BAP	MS+5.0 BAP	Excellent	4	Excellent multiple shoots formed
MS + 3.0 BAP	MS+0.5 GA_3	Good	4	Fairly good amount of shoots formed
MS + 3.0 BAP	MS+1.0 GA_3	Good	4	"

this rooting medium shoot development and root formation continued concurrently for the first few days and the plants became large in size. Complete plantlets of carnation were obtained by Roest & Bokelmann (1981) by subculturing the shoots on a medium containing 0.1 mg/l IAA, while the sucrose level was 2% with 0.5% agar.

iv) ***Field Transfer***: Subsequent to root induction the *in vitro* regenerated plantlets after attaining a height of 10 cm when transferred into pots showed that most of the plants could not survive due to high temperature during May-June. However, when the plantlets were transferred into pots during August-September, majority of the plants survived. MS supplemented with 2.0 mg/l BAP was superior for shoot induction compared to other combination (Table 1). Furthermore, MS fortified with 5.0 mg/l BAP induced prolific adventitious shoot formation within a culture period of 4 weeks (Table 2). Cultures which were previously raised on MS with 2.0 mg/l BAP. GA_3 although did not help in shoot induction but showed shoot elongation.

The results of the present study shows that higher bud proliferation rate of carnation could be attributed to high BAP concentration, which is 5 times more than the previous reports of Roest & Bokelmann (1981) and Siddiqui *et al.*, (1993).

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