JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

COMMERCIALLY FEASIBLE MICROPROPAGATION OF *Carnation caryophyllus*.L

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ABSTRACT

A Procedure for commercially feasible micropropagation of Carnation Caryophyllus was developed. For induction of Shoot and their proliferation four combinations (MS1,MS2,MS3 and MS4) were compared. MS media supplemented with BAP (1Mg/l) + Kn (0.5mg/l) or BAP (1mg/l) +Kn (1mg/l) resulted in shoot initiation and proliferation. The first medium also caused the production of amorphous achlorophyllous tissue at the base of the shoots to a greater extent than the second medium. Rooting was obtained upon transferring the shoots on MS half strength medium without and growth regulators. The rooted plantlets were transplanted to earthen pots containing a mixture of soil sand and compost (2:1:1),

Key words: Carnation , MS medium, Micropropagation, Sucrose,

INTRODUCTION

Carnation (Dianthus Caryophyllus L.) is an important flower crop having great commercial value as a cut flower. The importance of this ornamental flower is due to its beauty, diversity of colour, excellent keeping quality and wide range of different forms. (Ali *et al* 2008, Kanwar and Kumar, 2009). Carnation a member of the family Caryophyllaceae, and is a native of the Mediterranean region. The genus name comes from the writings of the Theophratus about Dias Anthas, the flower of Gods'.

Carnation is preferred to rose and chrysanthemum , in several escporting countries , due to its excellent keeping quality , wide range of forms, ability to with stand long distance transportation and remarkable ability to rehydrate after continuous shipping. From medical point of view, the carnation flower are considered to be cardiotonic, diaphoretic and alexiteric, Shiragur *et al.* 2004). Continuous development in production , imports and economic variables into account has raised the consumption of carnation in World's market up to 35 billion US Dollors in 2000 (Tarannum *et al.* 2014)

Carnation Flowers are sold as cut flowers round the year throughout the world and it is on the top three cut flowers traded in the international market. In the world, now it is estimated that more than 6000ha of land is under cultivation of carnation (Tarannum *et al* 2014). Considering the benefits of this crop and to fulfil the world's demand carnation breeders constantly seek new varieties with improved horticultural traits such as disease and past resistance and long vase life, Usually carnation varieties are maintained year after year by cutting on by other vegetative propagules (Karami 2008). In this way the plants remain same phenotype and genotype but they may become internally infeeted by pathogen like fungi, bacteria and viruses which decrease their field significantly vegetative propagation cannot eliminate the pathogen from the new plants. Plant tissue culture technique can play a key role to profuce large number disease free platlets which are True- to parental type parental type, Therefore *in vitro* technique is considered the best alternative method that may supply a large number of planting material for commercial planting and further studies. Although many researchers previously reported *in vitro* regeneration of carnation (Kakehi,1979; Leshem,1986 ; Millar *et al.*1991, Thakur *et al.*2002; As carnation has tremendous potential for growing under cover, high production coupled with excellent quality flowers, having demand both in foreign and domestic markets, the salient features of its cultivation under green house condition has led us to perform this research work.

MATERIAL AND METHODS

In this study the source material used as explants was taken from field grown tissue C.V Sam's Pride. Nodal explants after removing leaves that are over 5mm long are washed with Tween 20 for ½hr and then placed in 10 percent solution of bleach (0.525% sodium hypochlorite) for five minutes followed by three rinses in sterile distilled water for 5 min. Glass growth tubes each containing 10 ml of solid media were used for the initial phases of cultures and for subsequent proliferation phase glass bottles containing 50 ml of media were used.

Inoculation was carried out in laminar flow cabinet. It was cleaned by scrubbing with 70% ethanol solution and was irradiated with UV irradiations for 25 minutes before use.

MS medium (Murashige and Skoog's 1962) Supplemented with different concentration of auxin and cytokinin along with 3% sucrose was used Giving the following media (Table 1)

MS1- BAP (1 mg/l) + Kn (0.5mg/l)

MS2- BAP (1mg/l) + Kn (1mg/l)

MS3- BAP(2 mg/l) + Kn (0.5mg/l)

MS4- BAP (2 mg/l)+ Kn (1 mg/l)

In order to induce root system, shoots were excised individuall and culluered on half strenghth MS medium without any growth regulators. The pH of the media was adjusted to 5.8 before adding agar. All the cultures were maintained under light intensity of 2500-3000 lux having a temperature of 25± 1℃ and photoperiod was 16 hour with 8 hours dark period in every 24 hour cycle. Sub culturing was carried out every 4 weeks intervals. The well rooted plantlets were taken out from the test tubes and gently washed them to free from the medium. They were then transplanted to earthen pots containing a mixture of soil, sand compost. (Compost is a natural fertilizer made from cow dung) 2:1:1.

RESULTS AND DISCUSSIO

After the first week of culture a considerable swelling occurred in the explants on all the media. Four weeks after inoculation on media MS1 and MS2 same shoots started growing. MS3 and MS4 media also caused the induction of shoots but they appeared later than the explants cultured on MS1 and MS2 media.(Table1). In addition to a longes shoot inducation phase, fewer shoots per explants were observed on MS 3 and MS4 and development of the amorphous achlorophyllour tissue predominated. The initiation of culture on MS1 medium was similar to MS2, however more shoots were induced on MS2 media and the amorphous achlorophyllous tissue appeared less developed than on MS1. After the third period of subculture the difference between the behavior of the culture on both MS1 and MS2 media and those on media MS3 and MS4 media was more evident, the best shoot proliferation being archived in MS2 (Fig1D).

Due to low capacity of media MS3 and MS4 for shoot induction and shoot proliferation material grown on them was discarded the shoots clumps produced on the both MS1 and MS2 were subsequently subcultured every 4-6 weeks in a fresh medium. Rooting in the shoots was obtained upon transferring the shoots on MS half strength medium without addition of any growth regulators.

Nodal segments taken field grown tissue showed approximately 90% survival. Previous study reported that MS supplemented with 1.0mg/L BAP was most effective to highest number of shoot regeneration of carnation. (Hussey,1979.; Kim and Kang, 1986.; Kovac, 1995,; Thakur *et al*. 2002.; Pareek *et a*l. 2004, Ali *et al*. 2008)

In the present investigation, shoot bud proliferation has been achieved from nodal buds by addition of BAP and Kn both in the culture medium. The proliferation of multiple shoots was best on medium containing 1mg/l BAP and 1mg/l.Kn By this method a single shoot tip produced more than 300 well rooted plants after two or three subculture Each passage of subculture was of four to five weeks duration. Also an increased shoot multiplication rate has been achieved by addiction of BAP in the culture medium by (Roest and Bokelman, 1981, Ghose 1986). The proliferating buds elongated into shoots upon culturing on hormone free MS basal medium.

Time span is also reduced because the shoot initiation phase is not required on media optimized and the shoot, directly showed a higher rate of proliferation. Regular transfer of the well rooted plants to small pots and later their subsequent transfer to green house showed a satisfaction survival percentage, The plants retain their clonal identity in the open field and flowered normality. Davis *et al.*(1997) also observed no mutant out of approximately 1500 carnation propagated by axillary shoots .They noted multiple shoots on MS containing 10μm Kinetin and 1 μm NAA, Multiplication percentage however was lower. Rooting phase was achieved on transfer of shoots in peat pellets. We observed rooting upon transferring the shoots on MS half strength medium without addition of any growth regulators. This makes the system suitable for commercialization.

CONCLUSION

The increasing demand of high quality plants material for export and marketing competition in the ornamental plants has necessitated their true- to- type, disease free propagation, through tissue culture so for, straight production from tissue culture plants has always been reconsidered economically not feasible, because micropropagated plants are more expensive than traditionally propagated material, as a conseqvence, traditional cutting production of those species has never been endangered. However the availability of healthy mother plants is extremely useful to increase *in vivo* production rates, an area where the full potential of tissue culture could be exploited- The production of carnation in bulk could be greatly beneficial for floriculture. In our case for shoot induction MS medium with 3% sucrose and both BAP and kinetin combination gave best results. For rooting half strength MS medium without any growth regulators' gave best results.

Plant tissue culture technique can play a key role produce large number disease free plantlets which are true to parental type. Therefore *in vitro* technique is considered the best alternative method that may supply a large number of planting material for commercial planting and further studies.

ACKNOWLEDGEMENT

The authors are greatful to Eureka Agrotech, Ltd, Bani Road, Mohanlalganj , Lucknow for providing research facilities to carry this work at their lab.

Table: 1 Shoot initiation on media supplemented with different levels of growth regulators.

Media	Shoot initiation		Shoots proliferation
	After 4 Week	After 12 weeks	Shoots per inoculum
MS+ 3% Sucrose			
(1) BAP (1mg/l) + Kn (0.5mg/l).	4.1 ±2.5	17.3±0.12	32±0.5
(2) BAP (1mg/l) + Kn (1mg/l)	9.3 ±3.2	25.2±0.32	48±1.02
(3) BAP (1mg/l) + Kn (0.5mg/l)	None	4.7±1.7	nd
(4) BAP (2mg/l) + Kn (1mg/l)	None	3.3±1.4	nd

The data are presented as mean±S.E For shoot proliferation the data presented are for the end of the fourth and fifth sub- culture period and are measured across the word culture passage.

Nd-not determined.

Fig: Shoot initiation and shoot proliferation in *Dianthus caryophyllus* L. cultured on different media.

(a)Shoot proliferation in MS3 media after 4Weeks.

(b)Shoot in MS2 media after 4 Weeks

(C)Abundant shoot proliferation when shoot clumps were cultured on MS1 media after 45 days

(d)Abundant shoot proliferation when shoot clumps were cultured in MS2 media after 45 days.

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