# Influence of different growth regulators on the *in vitro* morphogenesis of an ornamental variety of carnation

**Kiss, Zs. O.[1] and Mándy, A.[2]**

[1]*University of Economic Sciences and Public Administration, Faculty of Horticultural Science, Dept. of Farm Management and Marketing, 31 Villányi St., H-1118 Budapest*
[2]*University of Economic Sciences and Public Administration, Faculty of Horticultural Science, Dept. of Floriculture and Dendrology, 35-43 Villányi St., H-1118 Budapest*

***Summary:*** Callus formation, as a prerequisite for the induction of somaclonal variability, was achieved successfully with certain molar ratios between 2,4-dichlorophenoxyacetic acid and benzyladenine. Regeneration of new plants from shoot apex meristems could be significantly improved by the combined addition of very low amounts of indolebutiric acid, benzyladenine and gibberelic acid, dissolved in the Murashige-Skoog nutrient medium. These *in vitro* treatments may contribute to a more efficient micropropagation of the Rimini variety of carnation.

***Key words:*** carnation, mericlones, morphogenesis, phytohormones

## Introduction

The developmental control of mericlones is largely based on the interactions between several growth regulator substances, and it is possible to externally influence this interaction by addition of synthetic or natural phytohormones. Auxin- and cytokinin-type regulators can usually stimulate histogenetic and organogenetic processes, and sometimes gibberelins also enhance the action of the formerly mentioned two categories of plant hormones. Carnation is one of the most widespread ornamental plants *(Schmidt* 2002), and the high number of similar individuals is mainly obtained by *in vitro* micropropagation. This method also enables the cultivation of healthy plantlets, lacking any kind of infection. The main inconvenience of this multiplication is that the young plants obtained in artificial media are very sensitive to the environmental stress conditions that normally occur in natural habitats *(Dudits & Heszky* 2000, *Tomos & Pritchard,* 1994). Disinfection, genetic control, developmental regulation and acclimation have to be achieved in a cumulative manner, and this makes very difficult the production of the desired plant material. This is the reason why at present many attempts are done in order to improve the efficiency of micropropagation of useful plant species *(Balla* 1993, *Dudits & Dohy* 1998).

The aim of this work was to present some possibilities to improve the mericlone regeneration, micropropagation and organogenetic potential of a rare variety of carnation with deep red flowers.

## Material and method

*In vitro* cultures was started from shoot apical meristems of the Rimini variety of carnation (*Dianthus caryophyllus* L.) isolated under axenic conditions and inoculated on sterile Murashige-Skoog (MS) nutrient medium gelified with agar-agar *(Frink & Halmágyi* 1999, *Kokas* 1990, *Parthier* 1989). The control was grown on pure MS medium, while the other experimental variants contained the following synthetic and natural growth regulators: a) 5 µM benzyladenine (BA), b) 1.5 µM naphtylacetic acid (NAA), 1.5 µM kinetin (K) and 100 mg $l^{-1}$ kazein (Kaz) as an organic nitrogen source, added to a MS medium without B-type vitamins, inositol and glycin, c) 3 µM gibberellic acid ($GA_3$), 4 µM indolebutiric acid (IBA) and 6 µM BA.

For the induction of callus formation in the *in vitro* cultures, the MS medium was supplemented with the following amounts of 2,4-dichlorophenoxiacetic acid (2,4-D as a synthetic auxin also used as a herbicide) and of benzyladenine: 1) 0.5 mg $l^{-1}$ 2,4-D + 2.0 mg $l^{-1}$ BA, 2) 1.0 mg $l^{-1}$ 2,4-D + 1.5 mg $l^{-1}$ BA, 3) 1.5 mg $l^{-1}$ 2,4-D + 1.0 mg $l^{-1}$ BA, 4) 2.0 mg $l^{-1}$ 2,4-D + 0.5 mg $l^{-1}$ BA.

The *in vitro* cultures were maintained for 25 days in a growth chamber at 22 °C, with a daily photoperiod of 16 hours illumination (with a light energy flux of 30 W $m^{-2}$, corresponding approximately to 3600 lx) and 8 hours darkness. Every experimental setup included 20 different samples *(Kiss* et al. 1989). Shoot length, rhyzogenetic capacity, number of internodes and callus formation were evaluated biometrically and statistically processed with the Student test. Acclimation of the *in vitro* regenerated plants to *ex vitro* conditions was performed in large pots with vermiculite as a solid substrate, in a greenhouse that was opened for longer and longer daily periods, in order to achieve a progressive habituation to the dry air *(Miller* et al. 1991, *Parthier* 1989).

## Results and discussion

Benzyladenine, together with the cytokinin produced in the meristematic cells, stimulated the rate of mitotic process, while the interaction of auxins and gibberelic acid led to an enhanced cell elongation in the internodes of the stem and in the developing young leaves. This means that under these conditions the new plantlets grow much faster, they reach maturity and produce flowers in a much shorter period of time. Addition of benzyladenine alone resulted in a moderate stimulation of shoot growth, while the development of the regenerated mericlones was inhibited by around 50% in the absence of B-type vitamins, even if the nutrient medium was supplemented with exogenic auxin (NAA), citokynin (K) and kazein. The shoot growth of the mericlones regenerated from the apical meristem was almost doubled, as compared to the control, on the culture medium enriched with a combination of gibberelin ($GA_3$), auxin (IBA) and cytokinin (BA). This supplementation with micromolar amounts of external growth stimulators, interacting with the endogenous hormones produced by the plant cells, resulted in a very significant enhancement of cell divisions and elongation processes *(Figure 1)*.

Cytokinins usually enhance callus induction because they stimulate cell divisions that lead to the formation of an undifferentiated cell mass. But the addition of 5 μM benzyladenine did not enhance significantly the callus formation, because it did not lead to an improved hormonal balance in the *in vitro* cultured meristematic cells. As compared to the control, the combination of $GA_3$, IBA and BA intensified the generation of callus, but not as much as the joint addition of NAA, kinetin and kazein did *(Figure 2)*.

It has to be mentioned that callus formation is a positive phenomenon if one intends to initiate new individual varieties by means of somaclonal variability, but it is an undesirable side-event if the goal is to obtain regenerants that have to be identical with the formerly selected mother plant [*Balla* 1993, *Dudits & Heszky* 2000, *Schmidt* 2002).

Induction of callus formation in cultures of the regenerated shoots, as a prerequisite for somaclonal variability and selection of new carnation types, was assayed with the combined addition of different amounts of 2,4-D and BA. Callus formation was mostly stimulated by 1.5 mg $l^{-1}$ 2,4-D and 1.0 mg $l^{-1}$ BA, and it was less enhanced by 0.5 mg $l^{-1}$ 2,4-D and 2.0 mg $l^{-1}$ BA. It is very difficult to comment these results because the concentrations of endogenous auxin and cytokinin are not known, but under the described conditions it seems that slightly increased ratios between the externally added 2,4-D and BA exhibit the most favorable influence on callus formation of the basal segment of stems *(Figure 3)*.

Root formation was not stimulated by either of the experimental setups, and it was mostly inhibited by addition of 5 μM benzyladenine as an extra amount of cytokinin. This results can be explained by the fact that the ratio between total auxin and cytokinin concentrations in the cells was modified in favor of the cytokinins, which stimulate

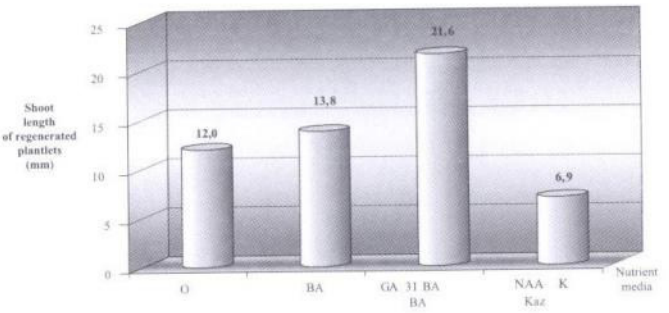


***Figure 1*** Effect of different growth regulators on shoot length of *in vitro* cultured carnation mericlones belonging to the Rimini variety (n=20, P<0.001)

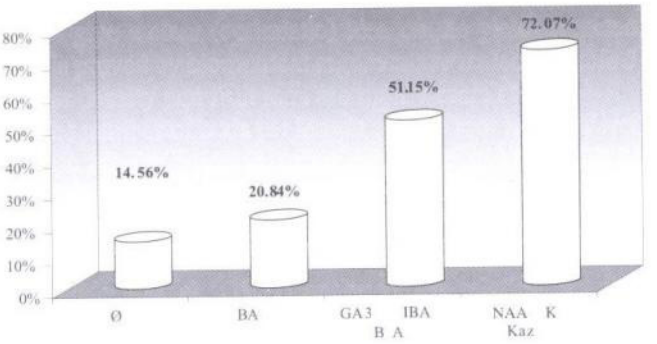


***Figure 2*** Influence of different combinations of growth regulator substances on the callus formation in shoot meristem cultures of the "Rimini" variety of carnation

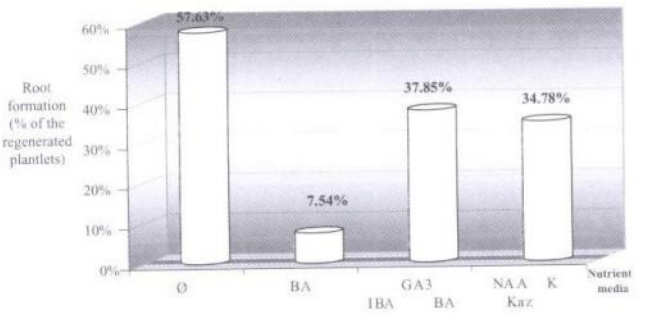


***Figure 3*** Influence of different auxin : cytokinin ratios on callus formation of carnation stem segments (n=20, P<0.001)

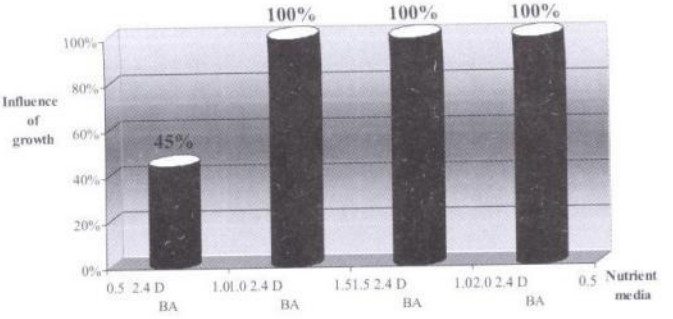


***Figure 4*** Influence of growth factors on the rhyzogenesis of *in vitro* regenerated plantlets of the "Rimini" variety of carnation

caulogenetic regeneration, while rhyzogenesis needs a higher auxin: cytokinin ratio *(Parthier* 1989). The slightest inhibition of root formation was observed in the medium supplemented with the combination of of 3 µM $GA_3$, 4 µM IBA and 6 µM BA *(Figure 4).*

The results show that the different organogenetic processes of the same plant require different hormonal conditions, a fact that makes the identification of an optimal medium for all of the developmental processes involved in the regeneration of entire plant organisms practically impossible.

## Conclusions

Shoot elongation of the regenerated, germ-free mericlones can be significantly stimulated with 3 µM gibberelic acid, 4 µM indolebutiric acid and 6 µM benzyladenine.

Instead of organogenesis, callus formation is enhanced by naphtylacetic acid, kinetin and kazein added to a MS medium without vitamins.

Root formation is mainly sustained by NAA and kinetin, and is mostly inhibited by 5 µM benzyladenine without being combined with exogenous auxins.

The callus formation needed for the induction of somaclonal variability is improved by moderately increased 2,4-D : BA ratios.

These results are also important from an economical point of view, as using the most optimal and effective medium to grow plants in can produce higher yields, which might make a project financially viable.

## References

**Balla, G. (1993):** A szegfű mikroszaporítása (in Jámborné [szerk.] Dísznövények Mikroszaporítása). Egyetemi jegyzet. Kertészeti és Élelmiszeripari Egyetem, Budapest.

**Dudits, D. & Dohy, J. (1998):** Biotechnológia: Lépéstartás Európával. Akadémiai Kiadó, Budapest.

**Dudits, D. & Heszky, L. (2000):** Növényi biotechnológia és géntechnológia. Agroinform, Budapest.

**Frink, J. & Halmágyi, A. (1999):** Természetes és mesterséges auxinok és citokininek hatásai a szegfű *in vitro* vegetatív fejlődésére. Múzeumi Füzetek. 8: 87–93.

**Kiss, E., Benczúr, E. & Gergely, Sz. (1989):** In vitro propagation of Hydrangea macrophylla. Kertészeti Egyetem Közleményei. 54: 101–105

**Kokas, Gy. (1990**): Szegfű az üvegházban és szabadföldön. Mezőgazdasági Kiadó, Budapest.

**Miller, R.M., Kaul, V., Hutchinson, J.F., Mekesweron, G. & Richard, D. (1991):** Shoot regeneration from fragmented flower buds of carnation (*Dianthus caryophyllus* L.). Annals of Botany. 68: 563–568.

**Parthier, B. (1989):** Hormone-induced alterations in plant gene expression. Biochem. Physiol. Pflantzen. 185: 289–314.

**Poething, R. S. (1990):** Phase change and the regulation of shoot morphogenesis in plants. Science. 250: 923–930.

**Schmidt G. (2002):** Növényházi szegfű termesztése. (in Schmidt (szerk.): Dísznövények termesztése). Mezőgazda Kiadó, Budapest.

**Tomos, D. & Pritchard, J. (1994):** Biophysical and biochemical control of cell expansion in roots and leaves. J. Exp. Bot. 45: 1721–1731.