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The influence of the genotype upon the *in vitro* and *in vivo* growth of greenhouse carnations

R. Sestras[1], E. Tamas[1], D. Pamfil[1], L. Mihalte[1],
A. Sestras[2], L. Chis[2] and C. Qin[3]

[1]University of Agricultural Sciences and Veterinary Medicine, Faculty of Horticulture, 3-5 Manastur St., 3400 Cluj-Napoca, Romania, e-mail: rsestras@usamvcluj.ro
[2]Horticultural Research Station, 3-5 Horticultorilor St., Cluj-Napoca, Romania
[3]School of Life Science, Shanghai University, Shanghai, 200436, China

Abstract. Carnations are familiar, widespread, and are among the most popular cut flowers. There is great diversity of greenhouse cultivars belonging to *Dianthus caryophyllus* L, habitually multiplied through cuttings or micro-propagation. In order to establish whether or not there is a connection between the vigour of the plants technically mature from the greenhouse and their *in vitro* growth, several traits of greenhouse carnation, grown both *in vivo* and *in vitro* were analysed in five cultivars (Polka, Tanga, Dark Tempo, Delphi and Indios). The influence of the genotype upon the vigour of the plants and upon the characteristics of the greenhouse flowers, as well as upon some features of the *in vitro* growth of the plantlets, was significant. The variability under *in vivo* conditions ranged from 5.0 to 17.8%, while the characteristics analysed *in vitro* showed a large span of variability values (*s%* = 7.9–51.0). Overall, the heritability showed high values for the analysed characteristics, both under *in vivo* (H^2 = 0.660–0.949) and *in vitro* (H^2 = 0.502–0.946) conditions. No statistically ensured correlations were recorded between the plant growth under *in vivo* conditions and of those with the same genotype under *in vitro* conditions; therefore the greater vigour of some genotypes from the greenhouse did not imply their more accentuated growth *in vitro*. The cultivars conspicuous for their superior characteristics will be used as genitors within the improvement programmes and also recommended to be tested for inclusion in the Official Catalogue of Plants.

Key words: carnation, cultivars, growth, *in vivo*, *in vitro*, variability, heritability

INTRODUCTION

Carnations are familiar, widespread, and are among the most popular cut flowers used in floral arrangements, corsages, and boutonnieres. There is great diversity of greenhouse cultivars belonging to the *Dianthus caryophyllus* L. species. A popular standard series, which is broadly considered to be the finest ever, includes the Sims, developed from the original William Sim cultivar (1938), named after the Maine breeder. A wealth of new cultivars are being created and tested for cropping by breeders around the world. Defining characteristics are an elegant deportment (influenced by the plant vigour, the stem height, the stem diameter, the foliage), a wonderful array of colours in addition to their shape, size, the number and shape of

petals, etc. The most valuable creations resulting from testing are multiplied and become part of the production process.

Along with the conventional vegetative multiplication of carnation that has been known and used for years, new *in vitro* methods have been developed lately both for industrial propagation (Pierick, 1987; Chu, 1992; Davis et al., 1993), and breeding purposes (Radojevici et al., 1990; Sestras et al., 2003).

Micropropagation represents a *modus operandi* for producing millions of genetically identical plants under controlled conditions. This method certifies indubitable advantages such as saving time and space, clone uniformity, freedom from seasonal constraints and prevention of the spread of new plant diseases. Tissue culture has been successfully employed for the multiplication of greenhouse carnations all over the world (Pamfil, 1991).

As with other species, it was noticed that in the case of greenhouse carnations, the genotype influences the success of the *in vitro* culture, as well as the evolution of the explants and the growth of plantlets (Sestras et al., 2004; Cristean, 2005). In order to identify the response of some new greenhouse carnation cultivars to the *in vitro* culture, research was launched to investigate a possible connection between the *in vitro* growth of plantlets and the growth and vigour of the plants from the greenhouse (*in vivo*).

MATERIALS AND METHODS

The biological material was represented by five cultivars of greenhouse carnation (*Dianthus caryophyllus* L.): Polka, Tanga, Dark Tempo, Delphi and Indios. The cultivars are relatively new and have not yet been included in the Official Catalogue of Plants from Romania. The particularities of the *in vivo* versus the *in vitro* growth of these carnation cultivars were studied at the Horticultural Research Station of Cluj-Napoca, Romania. The appraisal of the traits of the *in vivo* growth was performed in greenhouses on plants multiplied through cuttings, when stems and flowers were at commercial maturity. Five traits of the *in vitro* growth were analysed on four-week-old neo-plantlets obtained from the culture of apical meristem on a standard MS medium (Murashige & Skoog, 1962). Thirty (30) plants per cultivar were analysed under *in vivo* conditions and, respectively, 30 plantlets per cultivar under *in vitro* conditions, grouped by tens (10) per replication. The ANOVA statistics test was employed to identify the differences between the traits of the cultivars. Also, coefficients of variability and heritability for the analysed traits and coefficients of correlations between traits (Botez et al., 1995) were calculated.

RESULTS AND DISCUSSIONS

As seen in the data in Table 1, significant differences were recorded between the cultivars tested *in vivo*, regarding the plant vigour and the characteristics of the flower.

In comparison with the mean of the experiment (the control), it is worth mentioning that Indios cv. has rather short plants, Polka cv. has high plants, Tanga and Delphi cvs. have flowers with an obviously large diameter, Indios cv. has a tendency to develop more than two flowers/stem and Tanga cv. has significantly more petals/flower than the other tested cultivars.

Table 1. The mean values of the main growing traits and flowers' traits for five greenhouse carnation cultivars.

Cultivar	Plant height (cm)	Length of internodes (cm)	No of internodes/ plant	No of leaves/ stem	No of shoots/ plant	Length of stem (cm)	Height of flower bud (cm)	Flower diameter (cm)	No of flowers per plant	No of petals/ flower
Polka	120.5 ^{xxx}	7.4	18.3 $^{x\,x}$	37.4 ^{xx}	4.1	100.4	4.0	6.0	3.0	32.8 ^{o}
Tanga	96.7	7.2	14.8 ^{o}	32.8	3.8	85.3	3.0	6.4 ^{x}	2.4 ^{o}	44.2 ^{xxx}
Dark Tempo	101.2	7.1	17.1	33.4	4.5	98.0	3.2	6.3	3.6	34.3
Delphi	93.0	5.6 ^{ooo}	16.3	27.0 ^{ooo}	4.1	89.9	3.1	6.4 ^{x}	3.3	34.8
Indios	86.4 ^{ooo}	7.3	16.0	36.2 ^{x}	5.0 $^{(x)}$	83.4	2.9	5.7 ^{ooo}	3.9 ^{x}	36.1
Mean Control)	99.6	6.9	16.5	33.4	4.3	91.4	3.2	6.2	3.2	36.4
DS 5%	*5.5*	*0.7*	*1.4*	*2.8*	*0.8*	*12.6*	*1.0*	*0.2*	*0.7*	*2.8*
DS 1%	*7.4*	*0.9*	*1.8*	*3.7*	*1.0*	*16.8*	*1.3*	*0.3*	*1.0*	*3.7*
DS 0.1%	*9.6*	*1.2*	*1.4*	*4.8*	*1.4*	*21.9*	*1.7*	*0.5*	*1.2*	*4.9*

Table 2. The mean values of the main growing traits *in vitro* for five greenhouse carnation cultivars.

Cultivar	Plantlets height (cm)	Length of internodes (cm)	No of internodes/ plantlet	No of leaves/ stem	No of shoots/ plantlet	No of roots per plantlet	Length of roots (cm)
Polka	5.0	0.7	2.9	8.9 ^{o}	1.1	6.0	2.2
Tanga	5.2	0.7	2.9	15.1 ^{xxx}	2.2 ^{xx}	5.0	1.4 ^{o}
Dark Tempo	4.2	1.0	2.5	8.6 ^{o}	1.0	4.3	2.0
Delphi	4.8	0.7	3.1	11.4	1.0	4.5	2.8
Indios	5.1	0.7	3.0	9.7	0.6 ^{o}	3.9	2.7
Mean (Control)	4.8	0.8	2.9	10.7	1.2	4.7	2.2
DS 5%	*0.9*	*0.3*	*0.6*	*1.8*	*0.6*	*1.8*	*0.7*
DS 1%	*1.2*	*0.4*	*0.9*	*2.3*	*0.8*	*2.4*	*1.0*
DS0.1%	*1.6*	*0.6*	*1.1*	*3.0*	*1.1*	*3.1*	*1.3*

Explanations of signs for the differences.

P value	Significance of differences		
	Positive	Negative	Interpretation
< *DS 5%*	-	-	Non Significant (NS)
Between *DS 5%* and *DS 1%*	x	o	Significant
Between *DS 1%* and *DS 0.1%*	xx	oo	Distinct Significant
> *DS 0.1%*	xxx	ooo	Very Significant

Table 3. The values of the coefficients of variability (s%) and heritability (H2) for the traits of five greenhouse carnation cultivars.

Traits	Coefficient of variability (*s%*)		Coefficient of heritability in broad sense (H^2)	
	in vivo	*in vitro*	*in vivo*	*in vitro*
Plant height	13.0	8.2	0.928	0.657
Length of internodes	10.8	17.7	0.918	0.586
No of internodes/plant	7.9	7.9	0.835	0.502
No of leaves/stem	12.1	24.9	0.942	0.946
No of shoots/plant	10.8	51.0	0.752	0.886
Length of stem	8.3	-	0.754	-
Height of flower bud	13.6	-	0.660	-
Flower diameter	5.0	-	0.884	-
No of flowers/plant	17.8	-	0.853	-
No of petals/flower	12.3	-	0.949	-
No of roots/plantlet	-	17.0	-	0.626
Length of roots	-	25.6	-	0.839

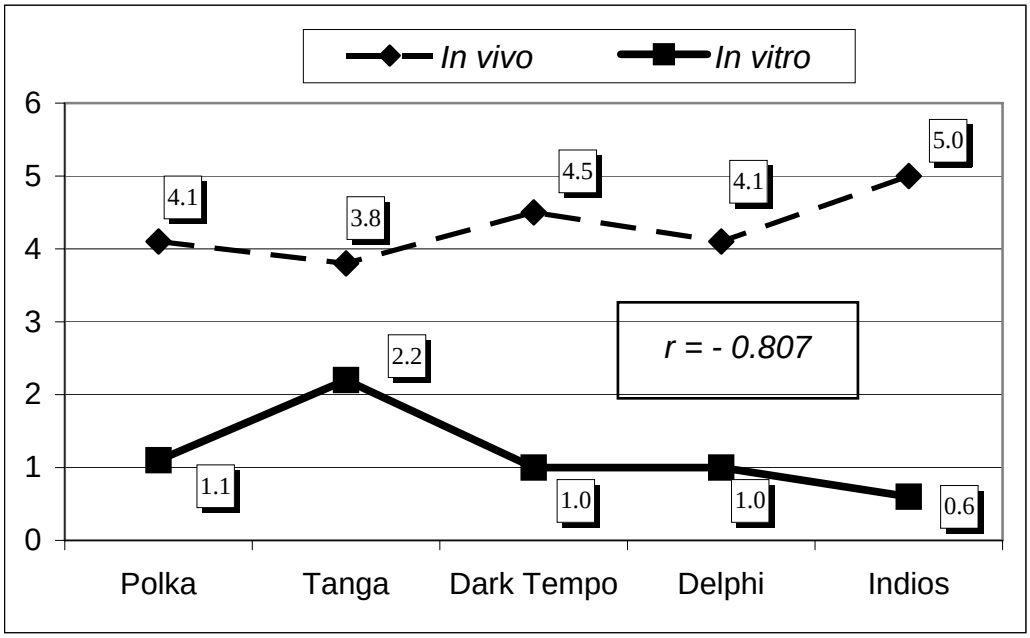

Fig. 1. The correlation between the number of shoots per plant (plantlets) *in vivo* and *in vitro* at five greenhouse carnation cultivars.

Table 4. The coefficients of correlation between the traits analysed *in vivo* at five greenhouse carnation cultivars.

Traits	Length of internodes	No internodes/ plant	No leaves/ plant	No of shoots/ plant	Length of stem	Height of flower bud	Flower diameter	No of flower/ plant	No of petals/ flower
Plant height	0.343	0.766	0.435	-0.432	0.852	0.967	0.043	–0.373	–0.374
Length of internodes		0.147	0.935	0.238	0.122	0.264	–0.523	–0.066	0.155
No internodes/plant			0.385	0.124	0.886	0.852	–0.271	0.290	–0.877
No leaves/plant				0.366	0.220	0.441	–0.733	0.108	–0.133
No of shoots/plant					–0.195	–0.307	–0.760	0.925	–0.419
Length of stem						0.827	0.145	–0.002	–0.656
Height of flower bud							–0.116	–0.225	–0.531
Flower diameter								–0.571	0.348
No of flower/plant									–0.649

r5% = 0.811 *r1% = 0.917* *r0.1% = 0.974*

Table 5. The coefficients of correlation between the traits analysed *in vitro* at five greenhouse carnation cultivars.

Traits	Length of internodes	No internodes/ plantlet	No leaves/ stem	No of shoots/ plantlet	No of roots/ plantlet	Length of roots
Plantlets height	–0.928	0.761	0.553	0.341	0.286	–0.062
Length of internodes		–0.932	–0.448	–0.167	–0.304	–0.217
No internodes/plantlet			0.363	–0.040	0.060	0.487
No leaves/stem				0.851	0.055	–0.526
No of shoots/plantlet					0.398	–0.863
No of roots/plantlet						–0.373

r5% = 0.811 *r1% = 0.917* *r0.1% = 0.974*

Besides these traits, the five cultivars were also assessed for their decorative value and the longevity of cut stems in water. On a scale from '0' (low value) to '5' (high value), all cultivars were recorded with average values over 4. The cultivars conspicuous for their valuable traits (high and straight flower stem**,** large flowers and a large number of petals/flower, superior decorative qualities) will also be tested for other traits (production, resistance to stress agents) and possibly recommended for inclusion in the Official Catalogue of Plants. At the same time, the cultivars exhibiting the most favourable traits will be used as genitors within new improvement programmes at Cluj-Napoca.

In the *in vitro* culture, the effect of the genotypes was obvious and significant for three of the seven traits analysed (Table 2): the number of leaves/stem, the number of shoots/plant and the root length. The number of leaves per stem varied from 8.6 (Dark Tempo) to 15.1 (Tanga); the number of shoots per plant, from 0.6 (Indios) to 2.2 (Tanga); the length of the roots, from 1.4 cm (Tanga) to 2.8 cm (Delphi).

The highest variability under *in vivo* conditions (Table 3) was recorded for the number of flowers/plant (17.8%) and the height of flower bud (13.6%), while *in vitro* the analysed characteristics showed an extremely large span of variability values (*s%)* between 7.9 for number of internodes/plant and 51.0 for number of shoots/plant.

The heritability overall presented high values for the analysed traits, both under *in vivo* (H^2 = 0.660–0.949) and *in vitro* (H^2 = 0.502–0.946) conditions.

The coefficients of correlation between the traits analysed in the greenhouse carnation cultivars, studied *in vivo* and *in vitro*, are displayed in Tables 4 and 5.

In vivo, strong correlations, both positive and negative, were identified among different traits of the mature plant in the greenhouse.

A strong positive correlation was identified between the following: plant height and stem length (0.852); plant height and flower bud height (0.967); internodes length and number of leaves/plant (0.935); number of internodes/plant and stem length (0.886); number of internodes/plant and flower bud height (0.852); number of shoots/plant and number of flowers/plant (0.925); stem length and flower bud height (0.827).

A strong negative correlation was identified between the number of internodes/ plant and the number of petals/flower (–0.877).

In vitro, the strongest positive correlations were computed between the plantlets height and the number of internodes/plantlet (0.761),and between the number of leaves/stem and the number of shoots/plantlet (0.851). Negative correlations were identified between the plantlets height and the internodes length (–0.928); between the internodes length and the number of internodes/plantlet (–0.932), and between the number of shoots/plantlets and the root length (–0.863).

Correlations between the desirable traits can be used as selection indices in carnation breeding.

In order to verify the hypothesis that in some greenhouse carnation genotypes there is a connection between the vigour of the plant growth *in vivo* and *in vitro* (Sestras et al., 2004), the coefficients of correlation between the joint growth elements examined in parallel at the five cultivars were determined.

There were no statistically ensured correlations recorded between the plants' height, the length of internodes, the number of internodes/plants, the number of leaves/stem, or the number of shoots/plant at the plants examined in parallel, *in vivo* or

in vitro. One unexpected correlation, which is negative and very close to the limit of significance (–0.807), was calculated between the number of shoots/plant *in vivo*, in mature plants, and the number of shoots/plantlets *in vitro*, in four-week-old neo-plantlets (Fig. 1). Based on the results obtained, it can be asserted that during the experiment the vigour of the plantlets grown on the MS medium, four weeks from the inoculation of the explants, was not correlated to the vigour of the technically mature plants from the greenhouse.

CONCLUSIONS

Within the experiment carried out upon five greenhouse carnation cultivars, the genotype significantly influenced both the vigour of the plant growth inside the greenhouse and the traits of the flowers. Under *in vitro* conditions, the genotype caused significant differences in the number of leaves/plantlets and the number of shoots/plantlets, therefore the response of greenhouse carnations to micro-propagation can be influenced by the genetic heritage of the cultivars.

The relatively high variability of some traits that are meaningful for the cropping and breeding of greenhouse carnations makes it possible to identify some potential genitors for future programmes creating new cultivars. Hence, the probable somaclonal variability can be manipulated under *in vitro* conditions.

The high heritability of some traits indicates the fact that, through a judicious selection of the genitors, new cultivars can be obtained which would possess the desired traits, transmitted from the parental forms.

No statistically ensured correlations were recorded between the plant growth under *in vivo* conditions and the growth of the plants with the same genotype under *in vitro* conditions; therefore the greater vigour of some genotypes from the greenhouse did not imply their more accentuated growth *in vitro.* The cultivars conspicuous for their superior characteristics will be used as genitors within the improvement programmes and also recommended to be tested for future inclusion in the Official Catalogue of Plants.

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