IN VITRO PREDICTION OF PLANT HEIGHT FOR *Chrysanthemum x grandiflorum* (Ramat.) Kitam

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A B S T R A C T

The height of nine chrysanthemum genotypes (*Chrysanthemum x grandiflorum* (Ramat.) Kitam.) was studied in *in vitro* and *in vivo* conditions. The aim of the study was to find out if it was possible to predict the stem height at the time of flowering in the greenhouse on the basis of *in vitro* development. It was found that the studied genotypes could be grouped according to their height *in vivo* and *in vitro* into three groups: with tall, medium, and short stems. The coefficients of correlation between mean, minimum and maximum heights *in vivo* and *in vitro* were positive, very high (0.94), and highly significant ($P<0.001$). It was concluded that the height of different chrysanthemum genotypes at flowering time *in vivo* could be predicted easily on the basis of their height after only four weeks of *in vitro* cultivation. This is important in the breeding programmes for early selection by height of vegetatively reproducing plants in the case when the methods of radiation mutagenesis and tissue culture are combined.

Key words: chrysanthemum, *in vitro*, *in vivo*, height, correlations

INTRODUCTION

Selection activities with chrysanthemum (*Chrysanthemum x grandiflorum* (Ramat.) Kitam.) were initiated in the Regional Center for Research and Extension Service for Floriculture (the former Institute of Floriculture – Negovan) in 1998. Several approaches were used for increasing genetic variation, especially for these varieties which are difficult to propagate by seeds (Zhenhua and Shouhe, 1995; Jerzy and Zalewska,

1987; Przybyła, 1996; Predieri et al., 1997). In particular, it was achieved by irradiation of different vegetative organs with ^{60}Co gamma rays (Nencheva, 2001) followed by *in vitro* techniques for the isolation and propagation of the mutants (Broertjes and Van Harten, 1978; Ahloowalia, 1992; Furuya, 1999).

Recently there has been a growing interest in pot chrysanthemum production. One way of obtaining dwarf chrysanthemums was the expression of a foreign GAI gene (Petty et al., 2001). Nevertheless, determination of some characteristics for early detection of the valuable genotypes on the basis of their phenotype could considerably shorten the selection process (Huitema et al., 1987). One possibility for early detection was found to be the *in vitro* cultures, where some differences in the internode length of chrysanthemum were well expressed (de Jong et al., 1987). It is possible, though, that some temporary changes could take place in the development of plantlets after their transfer from *in vitro* to *ex vitro* conditions (Pospíšilová et al., 1999). However, to the best of our knowledge, there are no comparative studies concerning the *in vitro* and *in vivo* height of the whole stems not only for chrysanthemums but for other ornamentals as well.

The aim of this study was to compare the *in vitro* and *in vivo* stem height of chrysanthemum genotypes for early characterization and selection for their height.

MATERIAL AND METHODS

The experiments were carried out with nine chrysanthemum genotypes with different flower colour: 'Westland Bronze' – standard, 'Mitko' and 'Milka' – two original cultivars created by radiation mutagenesis by the author, and six mutant clones newly selected by the author. Stem explants (Himstedt et al., 2001) with one node, originating from sample healthy plants of each genotype, were placed for cultivation in 20 cm high tubes on 10 ml of MS medium (Murashige and Skoog, 1962) with MS vitamins, 3% sucrose and 0.7% agar. The cultivation room conditions were: temperature 24°C, 16/8h day/night. After six weeks of *in vitro* cultivation the shoots were micropropagated by stem fragments with 1-2 leaves and were placed for growing in the same conditions to obtain clones with at least 20 plantlets each. Only the plantlets originating from the first apical fragments with 3-4 leaves and a height of 4-5 mm were used for studying the chrysanthemums' height. The height of the plantlets obtained was measured after 4 weeks in order to assess the possible differences between the genotypes. After repeated micropropagation the plantlets were rooted *in vitro* in a solid MS medium (see above) supplemented with 0.1 mgl^{-1} naphtalenacetic acid (NAA). They were acclimated in a peat for one month. The plants were then

planted during the last week of July in beds at a density of 36 plants per m^2 and were grown in a glasshouse. The temperature in the glasshouse was maintained at 6-8°C during the night and 14-24°C during the day with no additional lighting. At flowering time the plants were cut at 5-6 cm above the soil and their height was measured.

Twenty plants of each genotype were randomly chosen for *in vitro* and *in vivo* studies.

Each genotype was characterized *in vitro* and *in vivo* with five statistical parameters: the mean value of all measurements, their standard deviation (SD), coefficient of variation (CV), and the minimum and maximum values among the experimental plants. Only the differences between the mean values were measured by the t-test. Significance tests were not applied for the SD's and extreme values for each genotype. The productmoment (Pearson) correlations were calculated between the five parameters *in vivo* and the same values *in vitro*. All calculations were made with the SPSS set of programmes.

RESULTS

Characteristics of the development of the genotypes *in vitro*

After four weeks of *in vitro* cultivation the average height of the nine genotypes varied between 4.5 cm and 10 cm (Tab. 1). The tallest stems were found for 'Mitko', followed by a set of genotypes – 'Westland Bronze', 507, 1112, 1612 with a height of 8.03 cm, 8.43 cm, 8.62 cm, and 8.21 cm respectively, next being 'Milka' and 1617 with a height of 7.30 cm and 6.71 cm. The smallest height of 4.5 cm was found for the genotypes 3618 and 5201 (Tab. 1). Of a similar *in vitro* height were 'Westland Bronze' and 1612, 507 and 1112, as well as 507 and 1612. The height of all the other genotype pairs differed highly significantly ($P<0.001$). Closest in height were the two shortest genotypes – 3618 and 5201, which were the most desirable from the pot selection point of view, with no significant difference between them.

The variation within each genotype was measured by SD and CV. The CV varied from 3.7 to 9.6. It was the highest for the newly developed 5201 and the lowest for 1612. Some of the genotypes (5201, 507 and 3618) had relatively high variability while others ('Milka', 1112 and 1612) had low variability. These results showed that the grouping according to height similarity did not coincide with the grouping according to the variation.

The minimum and maximum values of the height seemed to rank the genotypes in a similar way to their corresponding average height. Therefore, the measurements could be used successfully for the characterization of the genotypes by their height *in vitro*. It is a matter of interest to find out whether the same or similar ranking could be observed for the *in vivo* height of the same genotypes.

T a b l e 1. Height of chrysanthemum plantlets *in vitro* [cm]

Genotype	Mean	SD	CV	Min	Max
Westland Bronze	8.03 a*	0.50	6.20	7.4	9.0
Mitko	10.01	0.71	7.12	8.7	11.0
Milka	7.30	0.38	5.16	6.5	7.9
No. 507	8.43 bc*	0.69	8.19	7.6	10.3
No. 1112	8.62 b**	0.44	5.15	7.8	9.5
No. 1612	8.21 ac**	0.31	3.74	7.5	8.6
No. 1617	6.71	0.45	6.66	6.0	7.7
No. 3618	4.57 d	0.36	7.97	4.3	5.5
No. 5201	4.53 d	0.44	9.64	3.8	5.5

The mean values followed by the same letter do not differ significantly
* Mean values differ significantly at P<0.05
** Mean values differ significantly at P<0.01
All the other mean values, which are not marked with any sign, differ significantly at P<0.001

Characteristics of the development of the genotypes *in vivo*

At the time of harvesting the average height of the stems varied between 46.9 and 112.8 cm for the different genotypes (Tab. 2). The highest mean height of the flower stems was measured for 'Mitko' (112.8 cm) followed by the means of 1112 (100.1 cm), 507 (88.6 cm), 'Westland Bronze' (81.4 cm), and 1612 (80.1 cm). Stems of a medium height were measured for 'Milka' (74.4 cm) and 1617 (73.3 cm), and the shortest genotypes were again 3618 (46.9 cm) and 5201 (53.3 cm). Of a similar height were the following two pairs of genotypes: 'Westland Bronze' and 1612; 'Milka' and 1617. The two shortest genotypes differed significantly (P<0.05) as well as 'Milka' and 1612 (P<0.01). All the differences between the other genotype pairs were highly significant (P<0.001).

In terms of variation, the lowest variation was found for 'Milka', 1617 and 1112, followed by medium variation for three genotypes – 5201, 507 and 1612. The highest variation was measured for 'Westland Bronze', 'Mitko' and 3618. The results showed that the genotypes were grouped in a different way by the mean height and by the variation (CV) within each genotype. In the experiment *in vivo* the minimum and maximum values followed the ranking of the genotypes according to their average height.

The comparison of the studied chrysanthemum genotypes showed that they differed considerably according to their mean height *in vitro* as well as *in vivo*. The extent to which this difference was maintained in both conditions was a matter of interest especially for the determination of the height of plants before they are planted in the soil.

T a b l e 2 . Height of flowering chrysanthemum plants *in vivo* [cm]

Genotype	Mean	SD	CV	Min	Max
Westland Bronze	81.40 a	5.84	7.18	70	90
Mitko	112.80	8.10	7.18	97	125
Milka	74.40 b**	3.57	4.80	67	80
No. 507	88.60	5.46	6.17	75	98
No. 1112	100.10	5.29	5.29	88	110
No. 1612	80.10 a**	4.96	6.19	70	88
No. 1617	73.30 b	3.85	5.26	65	80
No. 3618	46.90 *	4.37	9.31	43	58
No. 5201	53.30 *	3.21	6.02	47	58

The mean values followed by the same letter do not differ significantly
*Mean values differ significantly at P<0.05
**Mean values differ significantly at P<0.01
All the other mean values, which are not marked with any sign, differ significantly at P<0.001

Correlation between the stem height of the genotypes *in vitro* and *in vivo*

The coefficients of correlation between the five parameters measured *in vitro* and *in vivo* were significant (Tab. 3). They were very high and positive for the mean height (0.968, P<0.001), minimum height (0.940, P<0.001) and maximum height (0.957, P<0.001) and lower, but also high and positive, for the SD within each genotype (0.702, P<0.05). However, all the correlations with the CV were non-significant. These results clearly showed that the height of the genotypes *in vivo* could be successfully predicted on the basis of their height *in vitro*.

In the *in vitro* experiment the correlations of the mean height with the minimum and maximum height, and between the extreme heights were also positive, very high and significant (P<0.001). They were, however, to some extent lower with the parameters of variation (SD and CV). In the *in vivo* experiment the correlations between the three measurements of the height (average, minimum and maximum) were even higher (P<0.001) than the ones for the experiment *in vitro*.

The comparison of the development of the nine genotypes studied showed that they could be easily identified by height at an *in vitro* stage. Therefore, genotypes for special uses could be chosen for further commercial growing in glasshouse conditions.

DISCUSSION

The height of chrysanthemum plants *in vivo* was found to be affected by several factors. Jeong et al. (1996) discussed the influence of the differences between the day and night temperatures on the length of

T a b l e 3. Correlations between statistical parameters for chrysanthemum

Parameters		*In vitro*				*In vivo*				
		SD	CV	min	max	mean	SD	CV	min	max
In vitro	mean	.551	-.516	.994***	.978***	.968***	.792*	-.269	.962***	.962***
	SD	1	.420	.505	.685*	.624	.702*	.069	.585	.641
	CV		1	-.563	-.362	-.391	-.122	.352	-.427	-.370
	min			1	.971***	.945***	.781*	-.236	.940***	.943***
	max				1	.958***	.803**	-.236	.943***	.957***
In vivo	mean					1	.802**	-.297	.998***	.995***
	SD						1	.312	.782*	.845**
	CV							1	-.316	-.209
	min								1	.992***

***The correlation is significant at the 0.001 level
**The correlation is significant at the 0.01 level
*The correlation is significant at the 0.05 level

internodes and the whole *Mentha rotundifolia* plants. In a comparative study it was found that taking into account both temperatures, but not the difference, could better predict the height of chrysanthemum (Carvalho et al., 2002). In our study the same temperature was maintained for all the genotypes, so the observed differences could not be attributed to any differences in temperature.

The light spectrum could also influence the *in vivo* development of chrysanthemum plants (Reddy et al., 1996). All of the genotypes analysed in this study were cultivated in the same natural light conditions to eliminate this possible effect.

The position of axillary buds on the stem was found to influence further development of shoots in *Rosa hybrida*, giving advantage to those originating from the basal position (Bredmose and Hansen, 1996; Bredmose et al., 1999). Chen et al. (2001) reported a topophysic effect on the *in vitro* regeneration of *Adenophora triphylla*. They obtained the best results for internode explants from the middle part of the stem. All the chrysanthemum plants in our study originated from the very apical part of plantlets and were of a height of 0.5 cm to avoid any possible topophysic effects.

This comparative study of nine chrysanthemum genotypes showed that they could be classified into three groups according to their *in vitro* height (Tab. 1). The mean height varied between 4.5 cm and 10.0 cm. The tallest genotype was ‘Mitko’ and the shortest ones were 3618 and 5201. The other genotypes were of a medium height from 6.7 to 8.0 cm. These results demonstrated

that the genotypes included in the study belonged to groups of different heights. The grouping pattern on the basis of the maximum and minimum height within each genotype was the same. Again, the maximum height was the highest for 'Mitko' and the lowest for 3618 and 5201 (5.5 cm). The minimum values for the height of the plantlets followed the same pattern. All these results showed that the genotypes in the study varied according to their *in vitro* height which could be equally well recognized as tall, medium or short on the basis of their average, minimum or maximum height after four weeks of *in vitro* cultivation.

The variation within each genotype *in vitro* measured by the CV was from 3.74% to 9.64%. This variation did not seem to be related to the corresponding mean height. The shortest genotypes, which are of interest for future selection for pot chrysanthemums, showed a similar height but a different variation. This indicated that further *in vitro* selection for height could be more successful with the genotype 5201 because of its higher variability than with 3618.

In *in vivo* conditions (Tab. 2) the mean height of the flowering plants varied between 46.9 cm for 3618 and 112.8 cm for 'Mitko'. The tendency for the grouping of the genotypes into three categories was observed again: tall stem genotypes ('Mitko' and 1112), medium stem genotypes (507, 'Westland Bronze', 1612, 'Milka', 1617), and short stem genotypes (5201 and 3618). The distribution of the genotypes on the basis of their minimum and maximum height was also the same. This *in vivo* classification based on the stem height almost completely coincided with that based on the corresponding measurements *in vitro.* Very little is known about the genetics of the stem height of the chrysanthemum possibly because it is affected by various abiotic and biotic factors such as day length, light spectrum, temperature, nutrition, viruses, etc. (Bakker et al., 1995). In a study with seven varieties of a mean stem height from 85 cm to 118 cm the broad sense heritability was found to be very high – 0.781 (Kunigunda, 2004).

In this experiment the comparative results from the development of the nine genotypes studied *in vitro* and *in vivo* showed that their height after four weeks of growth *in vitro* could serve as a basis for predicting their future height at flowering time in the greenhouse. This result agrees with an earlier report that the differences between the internode length of seven chrysanthemum mutants observed *in vitro* were maintained *in vivo* (de Jong et al., 1987). In our experiment the mean variation (CV) of the genotypes in both conditions was similar – about 6%. The grouping of the genotypes based on their variation in *in vivo* and *in vitro* conditions suggested a positive relation, but it seemed to be lower compared to that based on the mean height.

The high, positive and significant correlation coefficients in *in vitro* conditions between the three measurements of the height – mean, minimum and maximum, showed that each of them could be used for the characterization of the genotypes according to their height (Tab. 3). In *in vivo* conditions these positive correlations were even higher, significant and exceeded 0.99. Therefore, the mean height could be recommended for use as a basic selection parameter of interest in chrysanthemum breeding. The correlations between the same measurements of the height in *in vivo* and *in vitro* conditions were also positive, very high (above 0.94) and significant ($P<0.001$). These correlations proved that there was a link between the height of four-week-old *in vitro* the plantlets and their height at the time of flowering. To our knowledge there have not been other publications so far about the correlations between the height of the whole chrysanthemum stems *in vitro* and *in vivo.*

In conclusion, for the nine chrysanthemum genotypes analysed in this study, the stem height at flowering time under greenhouse conditions could be predicted as early as after four weeks of *in vitro* cultivation. The average, minimum, and maximum height of plantlets *in vitro* were equally informative for the distinction of the chrysanthemum genotypes *in vivo*. Even at the phase of four-week-old plantlets it is possible to select clones (mutants) with a short, medium, or tall stem depending on the selection aim. This is important for the selection of vegetatively reproduced plants in the case when the methods of radiation mutagenesis and tissue cultures are combined in breeding programmes. However, the *in vivo* uniformity of the genotypes in respect of their height could not be predicted reliably on the basis of their *in vitro* characteristics and field tests are necessary. On the whole, the mutant genotypes 5201 and 3618, which were of a low height *in vitro*, were also of a low height *in vivo* and were, therefore, more suitable for pot culture, while the other seven genotypes studied were suitable for cut flowers.

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MOŻLIWOŚĆ OKREŚLENIA WYSOKOŚCI ROŚLIN *Chrysanthemum x grandiflorum* (Ramat.) Kitam UPRAWIANYCH *IN VIVO* PRZEZ KULTURY *IN VITRO*

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S T R E S Z C Z E N I E

Badano wysokość 9 genotypów chryzantem (*Chrysanthemum x grandiflorum* (Ramat.) Kitam) w warunkach *in vitro* i *in vivo*. Celem badań było określenie wysokości łodygi w czasie kwitnienia w szklarni na podstawie rozwoju *in vitro*. Wykazano, że badane genotypy mogły być pogrupowane *in vivo* i *in vitro* według wysokości na trzy grupy: z wysokimi, średnimi i niskimi łodygami. Współczynniki współzależności średniej, wysokości minimum i maksimum *in vitro* i *in vivo*, były pozytywne, bardzo wysokie (0.94). Stwierdzono, że wysokość różnych genotypów chryzantem, w czasie kwitnienia *in vivo*, mogła być łatwo identyfikowana na podstawie wysokości, tylko po 4 tygodniach uprawy *in vitro.* Ważne jest to dla programów hodowlanych, aby prowadzić wczesną selekcję dotyczącą wysokości wegetatywnie rozmnażanych roślin, np. w przypadku promieniotwórczej mutagenezy.

Słowa kluczowe: chryzantema, *in vitro*, *in vivo*, wysokość, współzależność