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THE EFFECT OF GENOTYPE ON *GERBERA* SHOOT MULTIPLICATION IN VITRO

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The multiplication rate of eleven gerbera cultivars was tested on a MS medium that contained 4.5% sucrose, 80 mg L^{-1} adenine sulphate, 100 mg L^{-1} L-tyrosine. 0.5 mg L^{-1} IAA and 2.0 mg L^{-1} N^6-benzyladenine (BA). The highest multiplication rates (8 shoots) per inoculum in a 4 week period were shown by cvs. Ansofie, Terracerise and Lablinel; cvs. Maria, Shanghai and Fresultane had moderate rates (6—7 shoots), and cvs. Croduro, Raisa, Fredigor, Terramaxima and Fredibel had the lowest rates (4—5 shoots). Plantlets rooted effectively in three weeks on MS salts with 3% sucrose and 2.0 mg L^{-1} IAA, and successfully acclimatized to the greenhouse conditions.

Introduction

Plant tissue culture has produced many important results for commercial applications, particularly for pathogen elimination and rapid clonal propagation in agriculture/horticulture, but also for variety improvement and secondary metabolite production (Murashige and Huang 1987).

Currently, hundreds of millions of plants are produced through micropagation, primarily in Western Europe, the United States and the Far East. However, microclonal propagation is a relatively complex, multistep process that depends on many factors. The success of initiation and the rate of propagation is determined by the genotype within a species, as well as environmental factors (Tormala and Oy 1990).

A tissue culture procedure has been proven to be commercially practical in *Gerbera* propagation (Murashige et al. 1974, Pierik et al. 1975, Wozniak et al. 1982, Soczek and Hempel 1988).

Murashige et al. (1974) attained clonal multiplication by cultivating shoot tips, 2—3 mm in size, on an MS medium supplemented with (mg L^{-1}): 0.5 IAA, 10 kinetin, 80 adenine sulphate and 100 L-tyrosine. High levels of kinetin stimulated the growth of young shoots from the axillary buds of the explant, while the exogenous IAA contributed to the vigorousness of cultures. By subculturing the shoots on a fresh medium every four weeks, the process of multiplication can be continued as long as needed to gain the desired number of plants.

Fully developed inflorescences (Pierik et al. 1975), or young capitula (Laliberté et al. 1985) were also used as initial material for *Gerbera* multiplication in vitro. Using that procedure, Preil et al. (1977) regenerated haploid plants on halved capitulum explants.

Pierik and Segers (1973) induced callus and adventitious roots on *Gerbera* midrib explants, while Hedtrich (1979) obtained adventitious bud regeneration on leaf explants of gerbera cv. Vulkan on a medium supplemented with N^6-benzyladenine (BA) and gibberellic acid (GA_3).

Nowadays, the procedure elaborated by Murashige et al. (1974) is used exclusively for the rapid clonal multiplication in the commercial production of gerbera.

Pierik et al. (1982), and Sauer et al. (1985) have shown that multiple shoot production of gerbera strongly depends on the cultivar. The determination of the shoot multiplication rate of a cultivar makes possible the accurate calculation of the time period required for the production of a certain number of plants. This information is important if a fairly accurate production programme is to be calculated.

The aim of our work was to determine the multiplication rate of some commercially important *Gerbera* cultivars in the micropropagation laboratory located in the Adriatic area of Split (Croatia). We tested eleven cultivars and found that they could be grouped into three classes based on their multiplication rates.

Material and Methods

Shoot tip explants were obtained from cloned *Gerbera jamesonii* Bolus plants grown in the greenhouse. The explants were excised from rhizome plants, 1—2 cm in size, stripped of roots, and young leaves were cut to 5—10 mm. After a one hour rinse in tap water, plant material was disinfected by immersion in 1—5% Izosan G solution, a chlorine product (Pliva, Zagreb), for 10—20 min, and by rinsing it in sterile distilled water (3x10 min).

The bud explants were further prepared under a stereo microscope (magnification 25x). The leaves arching over the apical dome were carefully broken off, while the 2—4 youngest leaf primordia were left in place. Thereupon, the shoot apices (0.5—2.0 mm) were excised together with up to 1 mm of the explant base. Apice explants and developed shoots in the first two subcultures were cultured individually on a 15 ml medium in test tubes (24x150 mm) covered with transparent plastic caps. In the subsequent subcultures, shoots were inoculated into 340 ml jars (5 shoots per jar) that contained 40 ml of medium.

After a culture period of four to six weeks, the regenerated multiple shoots in the primary cultures were cut into divisions and transferred individually onto fresh medium.

The initiation medium consisted of an agarified medium MS modified by Murashige et al. (1974), which contained 4.5% sucrose, and (mg L^{-1}): 1 BA, 0.3 IAA, 80 yeast-extract and 240 peptone. Yeast-extract and peptone were added to the primary cultures only for the detection of bacterial contamination. For the multiplication stage, the same solid medium MS, but supplemented with 2.0 mg L^{-1} BA and 0.5 mg L^{-1} IAA, was used. The pH was adjusted to 5.7 prior to adding agar and the media were sterilized by autoclaving at 0.122 MPa for 20 min. After each four-week multiplication cycle, the mean number of shoots per inoculum was counted.

During the rooting stage the MS basal medium was supplemented with 3% sucrose and 2.0 mg L^{-1} IAA.

The first experiment was conducted with the cultivars Croduro, Fredibel, Fredigor, Fresultane, Lablinel and Terramaxima, and was run from September/October 1987 to February/March 1988. The second experiment used the cultivars Ansofie, Croduro, Maria, Raisa, Shanghai and Terracerise, and was run from September 1990 to March 1991. Productivity was maintained for six or seven multiplication cycles, respectively.

The cultures were incubated in a temperature-controled culture room at 22—24°C during a 16 h light period at a light intensity of 50 Wm^{-2}, and at 21° C during a 8 h dark period. The light source was white fluorescent tubes (40W, 400—700 nm, TEŽ Zagreb).

Rooted plantles were transferred to the greenhouse and placed into a pre-sterilized mixture of perlite and peat (70% Agriperlite, 3—5 mm granulation and 30% peat, pH 6.5). During the first week, the plants were continuously kept under intermittent mist, and in the following week, this was reduced to five to six-times a day. Thereupon they were relocated within the greenhouse till flowering.

The mean number of shoots produced per culture was calculated over all inoculated divisions per subculture. At least one hundred, but often even more, replicate cultures were grown in each subculture for each cultivar. Data were analyzed by the analysis of variance, and statistical significance was determined using the t-test (Cochran and Cox 1953) and the LSD test.

Results

Shoot multiplication

The sterilization procedure we used resulted in 20—40% of the primary cultures being sterile. The percentage depended on the age of the donor plants; older plants offered a higher resistance to the sterilization procedure.

The shoot production of eleven cultivars was tested over two different periods of time. The cv. Croduro was estimated in both experiments and it showed a very similar multiplication rate (4.2, 4.4) in both periods (Figs. 1 and 2). In the first experiment (Fig. 1), Lablinel had a higher multiplication rate in relation to Croduro and Terramaxima, at a level of 5% confidence limits, and higher than Fredigor and Fredibel at a level

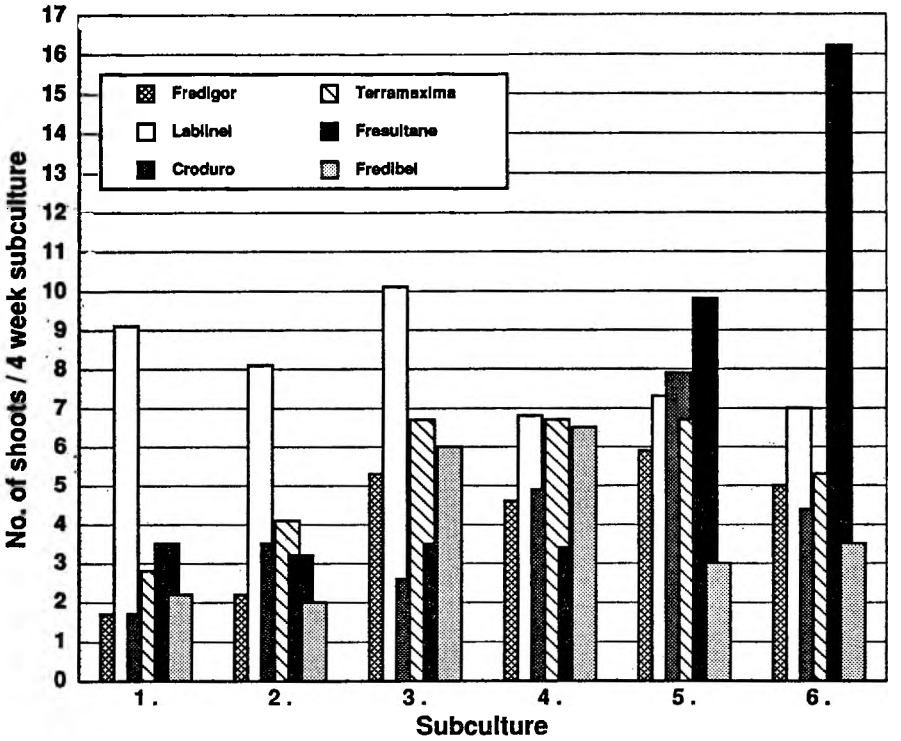

Fig. 1. Mean number of *Gerbera* shoots in vitro, tested from September/ /October 1987 to February/March 1988. Cultures grown on medium MS with 2.0 mg L^{-1} BA and 0.5 mg L^{-1} IAA. The sample size of any cultivar was 100 or more divisions per subculture.

of 1% confidence limits. Differences in shoot multiplication rates between other cultivars in the first experiment were not statistically significant (Table 1).

The second experiment (Fig. 2) showed that Ansofie and Terracerise had the highest rates of shoot multiplication (7.8, 8.3 respectively), cvs. Shanghai and Maria had moderate rates (6.5, 6.9), and Croduro and Raisa had the lowest rates (4.4, 4.9). The mean number of shoots obtained for Croduro and Raisa was statistically significant when compared to other cultivars (Table 1).

Based on our results, we have ranked the investigated cultivars into three classes: a) Ansofie, Terracerise and Lablinel with very high multiplication rates, b) Maria, Shanghai and Fresultane with moderate rates, and c) Croduro, Fredigor, Fredibel, Raisa and Terramaxima with the lowest multiplication rates.

Root formation in vitro and plant acclimatization

The shoots generated in the multiplication stage were transferred to the rooting medium, which consisted of a basal MS medium lacking adenine sulphate and L-tyrosine, but supplemented with 3% sucrose and 2 mg L^{-1} IAA. The intensity of light was the same as for the multiplication stage. Fifteen shoots per jar were inoculated and after three weeks, root formation in cultures was counted. Depending on the cultivar, the rooting percentage ranged from 72—99% (Table 2). Lower percentages of rooted shoots coincided with cultures contaminated by bacteria. Very severe contamination could even block the further growth of the generated roots. Therefore, we were not able to consider the influence of genotype on adventitious root induction.

After 15—20 days, rooted plantlets were transplanted to the greenhouse where they successfully acclimatized to ex vitro conditions and flowered. Among the roughly 20,000 plants of the tested cultivars, we have not noticed any phenotype abberations.

Discussion

For all the cultivars tested in our experiments, in the multiplication stage of culture we used the nutritive medium MS with 80 mg L^{-1} adenine sulphate, 100 mg L^{-1} L-tyrosine, 0.5 mg L^{-1} IAA and 2.0 mg L^{-1} BA.

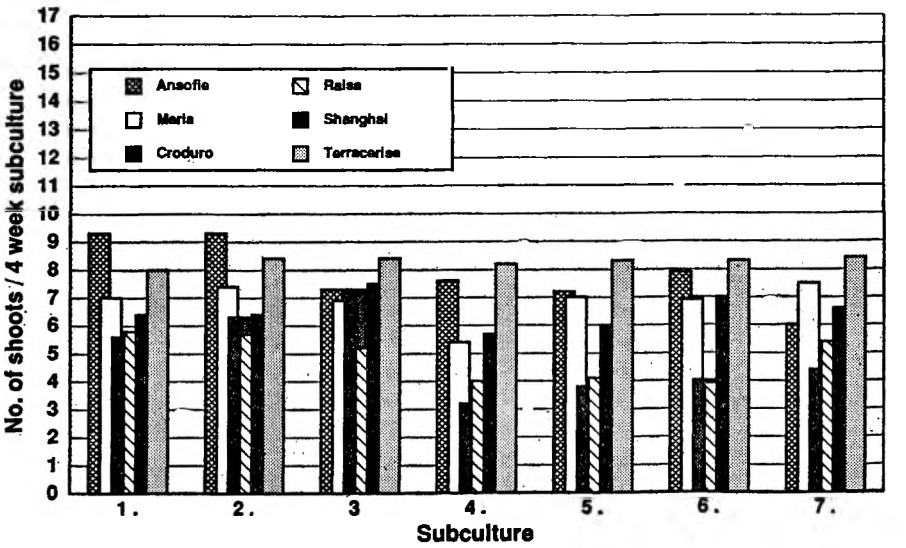

Fig. 2. Mean number of *Gerbera* shoots in vitro, tested from September 1990 to March 1991. Medium and sample size were the same as in Figure 1.

Table 1. Multiplication rates of the eleven tested *Gerbera* cultivars

Cultivar	Mark	Average shoot number		LSD
Experiment 1				
Fredigor	a	4.12	ab**	2.92
Lablinel	b	8.07		
Croduro	c	4.17	bc*	2.91
Terramaxima	d	5.38	bd*	2.11
Fresultane	e	6.60		
Fredibel	f	3.87	bf**	3.01
Experiment 2				
Ansofie	g	7.80	gh**	1.01
			gj**	1.37
Croduro	h	4.43	gk*	1.18
			hj**	1.01
Maria	i	6.87	hk**	1.47
			hl**	1.31
Raisa	j	4.87	ij**	0.79
			il**	0.73
Shanghai	k	6.51	jk**	1.01
			jl**	0.98
Terracerise	l	8.29	kl**	0.64

* Significant at 5% level, ** significant at 1% level using LSD test.

Table 2. Rooting (%) of *Gerbera* plantlets in two weeks on the medium MS supplemented with 3% sucrose and 2.0 mg L^{-1} IAA

Cultivar	Treated shoots No.	Rooted plantlets No.	%
Fredigor	2,161	1,611	74.5*
Lablinel	2,430	2,304	99.0
Croduro	7,116	6,525	91.7
Terramaxima	7,224	5,254	72.7
Fresultane	3,165	2,280	72.0
Fredibel	2,390	1,770	74.0*

* Cultures contaminated by bacteria

Reports published on micropropagation of gerbera show that kinetin, also in fairly high concentrations, was used most often as a cytokinin. Murashige et al. (1974) suggested 10 mg L^{-1} kinetin for optimal shoot formation, while Pierik et al. (1982), after testing ten cultivars, recommended a lower kinetin concentration (5.0 mg L^{-1}). Gregorini et al. (1976) studied the effect on the culture of *Gerbera* vegetative tips of the following different cytokinins: kinetin (10 mg L^{-1}), zeatin riboside (1.3 mg L^{-1}), and zeatin (1.6 mg L^{-1}) with added 0.5 mg L^{-1} IAA. They found zeatin riboside to be the most suitable for further subcultivation and multiplication of plantlets. Laliberté et al. (1985) used a medium very similar to ours, with the exception of a lower concentration of IAA (0.1 mg L^{-1}), and obtained an average shoot multiplication rate of 4.0 (cv. Pastourelle) and 6.0 (cv. Mardi Gras) in the first subculture.

Kinetin was considered most effective in adventitious shoot induction, at least in callus cultures of *Compositae* (Flick et al. 1983); Kothari and Chandra (1986), however, showed that in *Tagetes erecta* only BA was effective in caulogenesis. In our experiments, BA proved to be a good shoot bud inducer, and a concentration of 2.0 mg L^{-1} seemed to be sufficient for an acceptable rate of shoot multiplication. Plantlets obtained in our cultures did not show any leaf deformation or other unacceptable growth abnormalities during their development in the greenhouse.

The difference in shoot multiplication rates that we obtained in cultures of several cultivars supports the results of other authors (Pierik et al. 1982; Roest and Boekelmann 1975, 1981; Sauer et al. 1985).

On the basis of our results we are able to calculate precisely a plantlet production schedule for the cultivars tested and the period of time required to propagate a desired number of plants.

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SAŽETAK

UTJECAJ GENOTIPA NA UMNAŽANJE IZDANAKA GERBERE IN VITRO

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Istražen je utjecaj genetske osnove na stopu umnažanja jedanaest sorti gerbere na hranidbenoj podlozi MS s dodatkom 4,5% saharoze, 80 mg L^{-1} adenin sulfata, 100 mg L^{-1} L-tirozina, 0,5 mg L^{-1} IAA i 2,0 mg L^{-1} N^6-benziladenina (BA). Najvišu stopu multiplikacije po presadnici (8 izdanaka) u vremenu od 4 tjedna imale su sorte Ansofie, Terracerise i Lablinel; sorte Maria, Shanghai i Fresultane umjereno su se multiplicirale (6—7 izdanaka) a sorte Croduro, Raisa, Fredigor, Terramaxima i Fredibel imale su najslabiju stopu umnažanja (4—5 izdanaka). Biljčice su se u tri tjedna dobro zakorijenile na podlozi MS-soli s 3% saharoze i 2,0 mg L^{-1} IAA i uspješno prilagodile uvjetima u stakleniku.

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