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IN VITRO PLANT REGENERATION FROM STEM SEGMENTS OF SEVERAL CULTIVARS OF CHRYSANTHEMUM *(CHRYSANTHEMUM MORIFOLIUM* RAMAT.)

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Jevremović, S., Radojević, Lj. (1995): *In vitro plant regeneration from stem segments of several cultivars of chrysanthemum (Chrysanthemum morifolium Ramat.* – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 107 - 114.

Plant regeneration of *Chrysanthemum morifolium* Ramat. *cvs.*: „Fanshine Improved", „Pink Snowdon", „Klondike", „Yellow Spider", „Rivalry", „Crimson Robe", „Bronze Mundial" and „Tom Pierce" using nodal and internodal segments was obtained. Stem segments were cultured on MS mineral solution (Murashige and Skoog, 1962) containing 3% sucrose, 0.7% agar and (in mgL^{-1}): inositol 100, nicotinic acid 10, B_1 30, adenine sulphate 80 and tyrosine 100. This basal medium was supplemented with varying concentrations of indole 3-acetic acid (IAA, 0.1-0.5 mgL^{-1}) and benzyl aminopurine (BAP, 1 mgL^{-1}). Shoot multiplication takes place also on the same medium. Shoots yeild (19.2-90.0%) and average number of shoots *per* explant (2.4-8.6) were affected by the cultivar and medium. Micro shoots were on basal hormone free medium with 1% sucrose and 1/2 MS mineral solution successful rooted. Microplants 10-12 cm tall were transferred into pots, during spring, after 5-6 months plant flower evocation was observed in all plants.

Key words: *Chysanthemum morifolium* Ramat., micropropagation, stem segments culture
Ključne reči: *Chysanthemum morifolium* Ramat., mikropropagacija, kultura segmenata stabla

INTRODUCTION

Chrysanthemum morifolium Ramat. *(Asteraceae)* is a complex hybrid derived from several species that grow wild in China and Japan. This species is one of the three most important cut flowers in the world. Chysanthemums grown from seed are heterogeneous and are usually propagated by cuttings (Cathey, 1968). *In vitro* plant regeneration of Chrysanthemum has been reported earlier by Hill (1968), Roest and Bokelmann (1975), Sangwan et al., (1987), Lu et al., (1990), Bhattacharya et al., (1991) and others using different explants and media. There was no reports about *in vitro* tissue culture of chrisanthemum cultivars that we used in our experiments. This paper describes protocol for plant regeneration of 8 cultivares of chrysanthemum that has been commercially cultivated in our country.

MATERIALS AND METHODS

Stems of *Chrysanthemum morifolium* Ramat. *cvs.* „Fanshine Improved" („FI"), „Pink Snowdon" („PS"), „Klondike" („K"), „Yellow Spider" („YS"), „Rivalry" („R"), „Crimson Robe" („CR"), „Bronze Mundial" („BM") and „Tom Pierce" („TP") were prepared for tissue culture by method that were previously reported Radojević et al. (1987).

Nodal and internodal stem explants (0.3-0.5 cm) of flowered shoots were cultivated on A and B medium. Basal medium (BM) contained MS mineral solution (Murashige and Skoog, 1962), 3% sucrose 0.7% agar and (in mgL^{-1}): inositol 100, nicotinic acid 10, B_1 30, adenine sulphate 80 and tyrosine 100. This basal medium was supplemented with two concentrations of indole 3-acetic acid (IAA) and benzyl aminopurine (BAP). Medium A was BM medium supplemented with IAA 0.1 mgL^{-1} and BAP 1.0 mgL^{-1} and medium B was BM with IAA 0.5 mgL^{-1} and BAP 1 mgL^{-1}, pH 5.8. Shoot multiplication was on same media.

Shoot rooting was on C, D and E medium. The C medium contained MS mineral solution (Murashige and Skoog, 1962), 1% sucrose 0.7% agar and (in mgL^{-1}): inositol 100, nicotinic acid 10, pantotenic acid 10, B_1 2, B_6 1, adenine sulphate 80 and tyrosine 100, pH 5.8. Medium D was same as C only with 1/2 MS. Medium E was as medium C supplemented with NAA 0.02 mgL^{-1}. Rooted shoots of chrysanthemum were grown in greenhouse since flowering.

RESULTS AND DISCUSSION

Stem segments of chrysanthemum cultivars used in this work have different morphogenetic responses. Internodal segments first formed callus and then adventitious shoots (Fig. 1). Nodal segments develops axially buds while at the cutting sides callus appeared and then adventitious shoots are formed axailly (Fig. 2).

Morfogenetic responses of segments on A and B medium are represented in Tab. 1. It is evident from experimental results that the greatest morfogenetic response give *cv.* „CR" (90%) and the lowest 19.2 *cv.* „FI" on medium A (Fig. 3). Multiplication of shoots was by formation of axially and adventitious buds. Index of mutliplication is

represented also in Table 1. Medium A was better for propagation *cvs.* „FI", „PS", „K", „YW" and „TP" but, medium B was better for *cvs.* „R", „CR" and „BM" (Fig. 4, Fig. 5). W a n b u g u and R a n g a n (1981) has been reported that BAP in low concentrations induced multiple shoots and the presence of auxins did not significantly enhance the morfogenetic response. Previously, E a r l e and L a n g h a n s (1972) observed that higher concentrations of cytokinins favoured multiple shoot development. We used as cytokinin BAP in high concentration 1 mgL^{-1} and only in combination with auxin (IAA, 0.5 mgL^{-1}) desirable results are obtained (R a d o j e v i ć et al., (1994). L u et al. (1990) used NAA as auxin in multiplication medium and concentration of 1 mgL^{-1} was more effective than lower and higher concentrations (0.2, 0.5 and 2.0 mgL^{-1} NAA). Our results suggests that higher concentrations of IAA (0.5 mgL^{-1}) significantly increase multiplication (3.6) index of shoots only in *cv.* „R" on medium with 0.1 mgL^{-1} IAA to 8.6 on medium with 0.5 mgL^{-1} IAA. L u et al. (1990), after hormone treatment transferred explants to hormone free shoot elongation medium. In our case, this transfer is not needed, because plantlets grow fine on these combinations of auxins and cytokinin.

Tab. 1. – Effect of medium composition on morfogenetic response and shoot multiplication of chrysanthemum

CULTIVAR	MEDIUM (hormones in mgL^{-1}) A= BM + 0.1 IAA + 1.0 BAP B = BM + 0.5 IAA + 1.0 BAP	% of exsplants that forms shoots	multiplication index
"FI"	A	19.2	3.4 ± 1.4
	B	20.0	3.1 ± 0.5
"PS"	A	40.0	2.8 ± 0.6
	B	66.7	2.3 ± 0.2
"K"	A	50.0	3.4 ± 0.1
	B	70.0	2.8 ± 0.1
"YW"	A	50.0	4.4 ± 0.6
	B	30.0	3.6 ± 1.3
"R"	A	20.0	3.6 ± 1.2
	B	30.0	8.6 ± 2.3
"CR"	A	90.0	2.9 ± 0.3
	B	66.7	3.3 ± 1.0
"BM"	A	20.0	3.0 ± 0.7
	B	60.0	3.8 ± 2.2
"TP"	A	30.0	2.5 ± 0.2
	B	36.4	2.4 ± 0.4

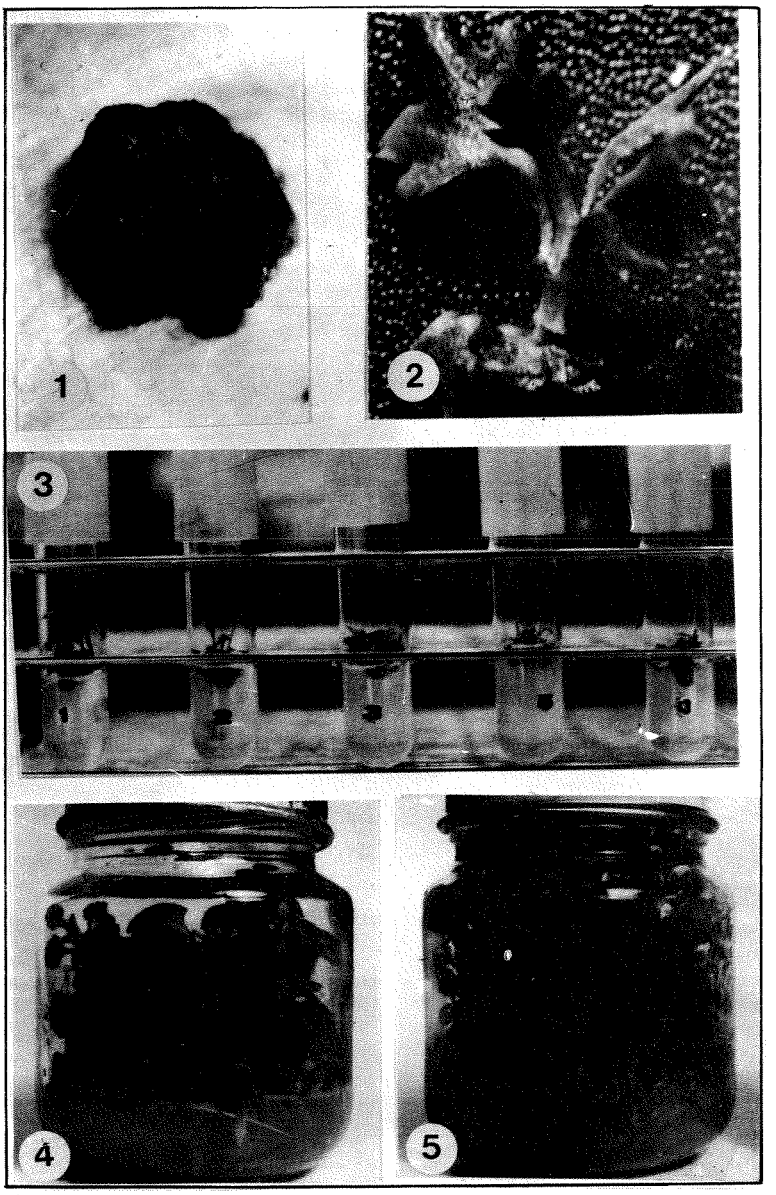
Microshoots (3-5 cm) were cultivated on rooting medium (C-E). The results obtained from three different rooting media (represented in Tab. 2) showed that number and length of roots depended of cultivar and medium. Rooting was the best

on D medium with 1/2 MS where number and length of roots was highest in all cultivars (Fig. 6). This results are similar as those described by B h a t t a c h a r y a et al. (1990) where half strenght MS medium was better than White's modified media. Medium supplemented with 0.02 mgL^{-1} NAA was proved to be less effective than MS hormone free medium.

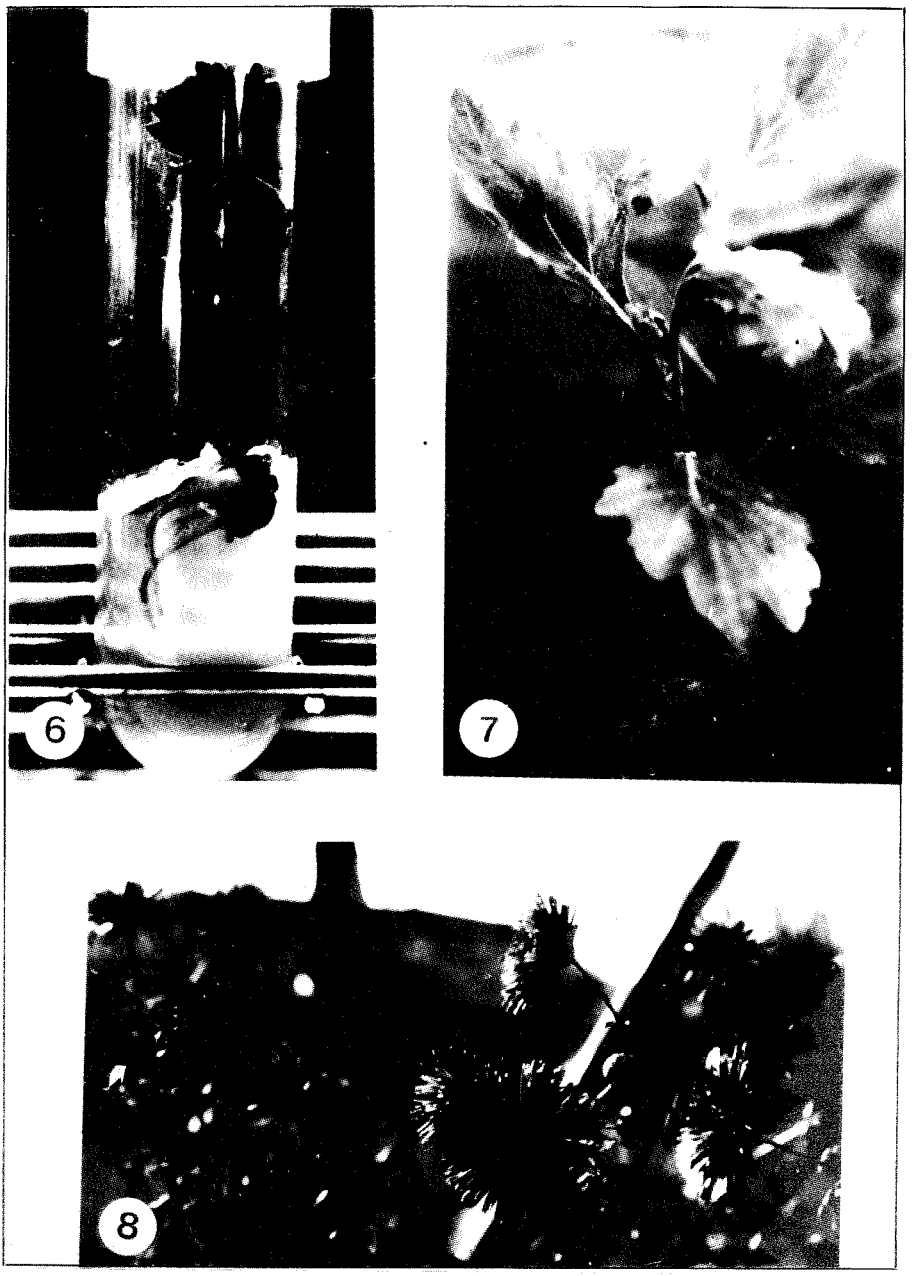
Tab. 2. – Chrysanthemum rooting on indicated media

CULTIVAR	MEDIUM (hormone in mgL^{1}) C = MS; D = 1/2 MS; E = MS + 0.02 NAA	Average N° of roots *per* plant	Average length of roots (mm)
"FI"	C	3.5 ± 2.5	72.3 ± 17.4
	D	4.3 ± 1.9	175.7 ± 63.9
	E	4.1 ± 2.4	82.3 ± 42.8
"PS"	C	7.4 ± 1.9	67.3 ± 27.3
	D	12.6 ± 3.5	120.4 ± 20.8
	E	9.5 ± 3.2	97.5 ± 20.6
"K"	C	2.6 ± 1.3	91.7 ± 56.9
	D	6.8 ± 3.2	123.8 ± 40.1
	E	5.1 ± 2.8	110.5 ± 48.8
"YW"	C	3.4 ± 1.0	52.6 ± 16.7
	D	3.5 ± 1.2	117.5 ± 23.8
	E	2.0 ± 0.8	75.5 ±15.1
"R"	C	1.0 ± 0.3	6.0 ± 2.1
	D	3.2 ± 1.1	82.2 ± 17.2
	E	1.2 ± 0.5	8.3 ± 2.3
"CR"	C	4.4 ± 2.5	61.5 ± 28.4
	D	5.7 ± 2.2	133.3 ± 29.3
	E	4.6 ±2.8	56.5 ± 22.2
"BM"	C	6.4 ± 3.5	16.2 ± 9.6
	D	4.8 ± 2.2	151.7 ± 8.5
	E	3.3 ± 2.4	32.4 ± 19.8
"TP"	C	8.7 ± 2.4	48.3 ± 10.5
	D	16.5 ± 2.6	75.8 ± 51.8
	E	8.4 ± 2.7	62.0 ± 31.5

Microplants about 10-12 cm tall were transferred into pots, during spring. Acclimatisation was achieved in *cv.* „FI"100% *cv.* „PS" 95%, *cv.* „K" 100%, *cv.* „YW" 98%, *cv.* „R" 96.5%, *cv.* „CR" 95.3%, *cv.* „BM" 98% and *cv.* „TP" 99% (Fig. 7). Plant development was normal and phase-change was induced after 5-6 mounts as seedling plantlets (Fig. 8). Phenotypic characters were the same as the donor explants.



Figs. 1.–5. – Micropropagation of *Chrysanthemum morifolium* in stem segments culture: 1. – Calus formation of internodal segments *cv.* „TP" 7-days in culture; 2. – Axially bud of *cv.* „TP" developed on internodal segment 7-days in culture; 3. – Stem segments of chrysanthemums *cvs.* „FI" (1), „PS" (2), „K" (3), „YW" (5) and „BM" (13) one mounth after initiation of culture; 4. – Shoot multiplication of *cv.* „Tom Pierce" on A medium (BM + 1 mgL^{-1} IAA + 1 mgL^{-1} BAP); 5. – Shoot multiplication of *cv.* „Rivarly" on B medium (BM + 0.5 mgL^{-1} IAA + 1 mgL^{-1} BAP)



Figs. 6. - 8. – Micropropagation of *Chrysanthemum morifolium* in stem segments culture: 6. – Shoot rooting of chrysanthemum *cv.* „TP" on D medium (MS 1/2); 7. – Acclimatized plant of *cv.* „FI"; 8. – Flowered plants of chrysanthemum *cv.* „K" in greenhouse

In present paper, we reported efficient, plant regeneration protocol of 8 cultivars of chrysanthemum by culture of stem segments. In conclusion, on this paper a new and efficient micropropagation protocol for *cv.* „FI", *cv.* „PS", *cv.* „K", *cv.* „YW", *cv.* „R", *cv.* „CR", *cv.* „BM", and *cv.* „TP" cultivars are achieved, being, the method also a new alternative for measurement the propagation procedure for *cv.* „FI", *cv.* „PS", *cv.* „K", *cv.* „YW", *cv.* „R", *cv.* „DR", *cv.* „BM" and *cv.* „TP" which has been previously described. The large numbers of shoots produced *per* explant and uniformity of regenerating plants make this system an ideal tool for chrysanthemum propagation, and a promising system for cryopreservation and the genetic manipulations.

REFERENCES

Bhattacharya, P., Dey, S., Das, N. & Bhattacharya, B. Ch. (1990): Rapid mass propagation of *Chrysanthemum morifolium* by callus derived from stem and leaf explants. – Plant Cell Rep. *9*: 439-442.

Cathey, H.B. (1969): *Chrysanthemum morifolium* (Ramat.) Hemsl. *In* The induciton of Flowering (I.T. Evans, ed) pp. 269-290, Mac Millan of Australia.

Earle, E.D. & Langhans, R.W. (1974): Propagation of Chrysanthemum *in vitro*. I. Multiple plantlets from shoot tips and the establishment of tissue cultures. – J. Amer. Soc. Hort. Sci. *99*: 128-132.

Hill, G.P. (1968): Shoot formation in tissue cultures of *chrysanthemum* „Bronze Pride". – Physiol. Plantarum *21*: 386-389.

Lu, C., Nugent, G. & Wardley, T. (1990): Efficient, direct plant regeneration from stem segments of chrysanthemum (*Chrysanthemum morifolium* Ramat. *cv.* Royal Purpure). – Plant Cell Rep. *8*: 733-736.

Murashige, T. & Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco cultures. – Physiol. Plantarum *15*: 473-497.

Radojević, Lj., Đorđević, N. & Mančeva, L. (1987): Clonal multiplication of gerbera by *in vitro* culture of different tissues. – Sym. Florizel 87 Arlon – Belgium. Plant micropropagation in Horticultural Industries. pp. 218-223.

Radojević, Lj., Marinković, N., Zdravković-Korać, S. & Jevremović, S. (1994): Primena različitih postupaka *in vitro* kulture u mikropropagaciji aftičke ljubičice, hrizanteme i karanfila. – Savremena poljoprivreda *42*(6): 117-127.

Roest, S. & Bokelmann, G.S. (1975): Vegetative propagation of *Chrysanthemum morifolium* Ram. – Sci. Hort. *3*: 317-330.

Sangwan, R.S., Detrez, C. & Sangwan-Norrel, B.C. (1987): *In vitro* culture of shoot tip meristems in some higher plants. – Acta Hortic. *212*: 661-666.

Wambugu, F.M. & Rangan, T.S. (1981): *In vitro* multiplication of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.) by micropropagation. – Plant Science Letters *22*: 219-226.

Rezime

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REGENERACIJA BILJAKA PRIMENOM KULTURE *IN VITRO* SEGMENATA STABLA KOD NEKOLIKO KULTIVARA HRIZANTEME (*CHRYSANTHEMUM MORIFOLIUM* RAMAT.)

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Proučavana je regeneracija biljaka *Chrysanthemum morifolium* Ramat. cvs.: „Fanshine Improved", „Pink Snowdon", „Klondike", „Wellow Spider", „Rivalry",

„Crimson Robe", „Bronze Mundial" i „Tom Pierce" u kulturi nodalnih i internodalnih segmenata stabla. Eksplanti su gajeni na MS hranljivoj podlozi sa mineralnim rastvorom Murashige i Skoog, (1962) 3% saharozom, 0,7% agarom i (u mgL^{-1}): inozitol 100, nikotinska kiselina 10, B_1 30, adenin sulfat 80 i tirozin 100, indol 3-sirćetna kiselina (IAA, 0,1-0,5 mgL^{-1}) i benzil aminopurin (BAP, 1 mgL^{-1}). Umnožavanje izdanaka je postignuto na istoj MS hranljivoj podlozi. Morfogenetski odgovor eksplantata (19,2-90,0%) kao i prosečan broj izdanaka po eksplantatu (2.4-8,6) zavisili su od kultivara i hranljive podloge. Najbolje oživljavanje „mikro" izdanaka je bilo na MS podlozi bez hormona sa 1% saharozom. Biljke, veličine 10-12 cm, odgajane su u uslovima staklare. Aklimatizacija „mikro" biljaka se odvijala u proleće i iznosila je 95-100% u zavisnosti od sorte. Posle 5-6 meseci biljke hrizanteme su cvetale i imale su istu boju cveta kao biljke donori.