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REGENERATION OF SOMATIC EMBRYOS FROM *in vitro* ISOLATED LIGULATE FLORETS OF CHRYSANTHEMUM

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Abstract. Many chrysanthemum mutants are chimeras built from tissues of a varied genetic composition. Regeneration *in vitro* of somatic embryos from the whole mutated ligulate florets or only from their small mutated fragments can lead to the separation of chimera components and, as a result, producing a new original cultivar. There was determined the effect of growth regulators (2,4-D; KIN or BAP) and explant type (whole or transversely-cut-into-half ligulate florets) on the efficiency of somatic embryogenesis of *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. 'Cool Time'. For the induction of the somatic embryogenesis ant the regeneration of somatic embryos the MS medium [Murashige and Skoog 1962] with 18.08 μM 2,4-D as well as with this auxin and 4.44; 8.88; 22.20 μM BAP or 4.65; 9.30; 23.25 μM KIN was used. The best results were obtained when transversely-cut-into-half ligulate florets were inoculated onto the medium with 4.65 μM KIN and 18.08 μM 2,4-D. This results may increase the probability of success in separation of chimera components in chrysanthemum breeding.

Key words: *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam., somatic embryogenesis, growth regulators

INTRODUCTION

Chrysanthemum × *grandiflorum* (Ramat.) Kitam. is a species highly appreciated by ornamental plants producers and breeders. However, it is estimated that about half of chrysanthemum cultivars are periclinal chimeras. As for morphology, they do not differ from genetically homogenous cultivars, however, problems appear if their components separate from each other, which is of special importance in the laboratory production of microcuttings. It is so since chimeras are the plants built from genetically-different tissues [Broertjes and van Harten 1988] and to maintain their stability, it is necessary to apply propagation with the use of meristematic explants. Their formation also consid-

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erably limits mutation breeding [Chakrabarty et al. 1999]. Many spontaneous or induced inflorescence colour mutations in *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. appear on a few whole ligulate florets or only in a form of a spot or stripe from one to a few thousand cells in size [Stewart and Dermen 1970, Malaure et al. 1991, Chakrabarty and Datta 2010]. Due to a small area of the tissues, such mutations are lost since they cannot get isolated and preserved using common methods of chimera components separation from leaf explants by adventitious shoot regeneration [Zalewska et al. 2007]. For that reason it is necessary to use other kinds of explants which contain such a genetic change and more efficient regeneration methods which facilitate the restitution of the plants changed. Tanaka et al. [2000] confirmed the usefulness of ray (ligulate) florets as an alternative source of explants for the regeneration of somatic embryos in chrysanthemum. Somatic embryogenesis, which is currently the most efficient method of plant regeneration, allows for plant restitution from single explant cells [Gahan and George 2008]. Somatic embryos, unlike the adventitious shoot, undergo successive development stages of embryogenesis, similar to the development of zygotic embryos [Dodeman et al. 1997].

The somatic embryo, similarly as the adventitious shoot, gets regenerated from a single somatic cell of the explant [Broertjes et al. 1976, Pavingerová et al. 1994, Gahan and George 2008]. The separation of chimera components, especially the sectorial ones, could thus occur through the regeneration of somatic embryos from the whole ligulate florets or only from their fragments. The somatic embryos regenerating from single cells of histogenic layer L1 or L2 of the ligulate floret, will represent the genotype of layer L1 or L2, respectively, and they will be built only from genetically homogenous tissues.

The induction of somatic embryogenesis in chrysanthemums in cultures *in vitro* is affected e.g. by such factors as: growth regulators [May and Trigiano 1991, Tanaka et al. 2000, Mandal and Datta 2005] and the genotype [Pavingerová et al. 1994]. The efficiency of regeneration is also enhanced by the division of the explant, which is connected with an intensified inflow, into the areas of the cutting places, of endogenous growth regulators as well as with an intensive uptake of exogenous growth regulators and the proliferation of the callus tissue [Gahan and George 2008]. The genetic stability of chrysanthemums derived from somatic embryos regenerated on media with relatively high concentration of auxin 2,4-D (18.08 μM) and different content of cytokinins (from 4.65 to 23.25 μM KIN or from 4.44 to 22.20 μM BAP) was confirmed with flow cytometry for 'Richmond' cultivar by Lema-Rumińska and Śliwińska [2009].

Both the regeneration through adventitious shoots and somatic embryos allows for the restitution of genetically homogenous plants with the phenotype changed as a result of breeding. However, a potential efficiency of somatic embryogenesis, facilitating the regeneration from each living somatic cell, gives advantage over the regeneration through adventitious shoots since it offers a chance for the mutations which appear only on an inconsiderable area of tissues to get identified.

The aim of the research was to determine the effect of growth regulators and the kind of the floret explant (the whole or a cut) on the regeneration of somatic embryos in *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. 'Cool Time' in terms of using that regeneration method for breeding.

MATERIAL AND METHODS

Culture of donor plants. For the research *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. 'Cool Time' pot cultivar, with half-full inflorescences with a green and yellow disk of tubular florets and white ligulate florets was selected. Method of culture of donor plants in greenhouse was chosen based on the previous conducted research [Tymoszuk and Zalewska, 2014].

Disinfection of explants. The ligulate florets were sampled from completely open inflorescences. Two whorls of tubular florets produced pollen. There was used the method of disinfection according to Tymoszuk and Zalewska [in print]. Both whole and transversely-cut-into-half ligulate florets were inoculated.

Culture initiation. The MS [Murashige and Skoog 1962] medium supplemented with 18.08 µM 2,4-dichlorophenoxyacetic acid (2,4-D), as well as with 18.08 µM 2,4-D and 4.65; 9.30; 23.25 µM kinetin (KIN) or 4.44; 8.88; 22.20 µM 6-benzylaminopurine (BAP) was used to initiate embryogenesis and to regenerate somatic embryos [Lema-Rumińska 2012]. The MS medium was additionally modified by increasing the content of calcium and iron by half. The medium included 3% (w/v) of sucrose and was solidified with 1.2% (w/v) Purified Lab-Agar™ provided by Biocorp. Prior to autoclaving, pH of the medium was set at the level of 5.8. The 350 ml jars were poured with 30 ml of the medium. One ligulate floret was inoculated into a jar.

Culture conditions. Cultures *in vitro* were maintained in the growth room at the temperature of 24 ±2ºC, exposed to a 24-hour photoperiod (16 hours of light, 8 hours of dark), using fluorescent lamps Philips TLD 36W/54 emitting daylight. The quantum irradiation intensity was set up at 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Observations and the method of the analysis of variance. For 10 successive weeks observations were made into the regeneration of callus tissue. After this time under the stereoscopic microscope MS-Z TRI provided by Precoptic at magnification from 0.7 × 10 to 4.5 × 10 embryos were isolated from callus tissue regenerated on explants. There were defined the number of embryos and their development stages. There were also defined the number of adventitious roots.

The experiment was set up in a completely randomised design. For each of the 14 experimental treatments 20 replications were applied, one explant for each. The effect of growth regulators contained in the medium and the explant type applied on the embryos regeneration was defined quantitatively by calculating the mean number of embryos per explant inoculated, as well as the mean number of embryos in the different development stages per explant. There was also calculated the percentage share of the explants regenerating embryos. Results for mean number of adventitious roots per explant inoculated and the percentage share of the explants regenerating adventitious roots were also statistically verified. The method of the analysis of variance was used and means for the treatment were evaluated with the Newman-Keuls test at the significance level of $P = 0.05$.

RESULTS AND DISCUSSION

Starting from the second week of cultures *in vitro*, the regeneration of green callus tissue on explants was observed. Callus appeared on the surface of all ligulate florets, both whole and transversely-cut-into-half, irrespective of the composition of growth regulators in the medium applied. Callus proliferation was most intensive around the ovary as well as in the cutting places. With the successive observation there was observed browning of the surface of the corolla. After yet another week, the callus was becoming cream-yellow-green in colour, and in some areas – even reddish.

Somatic embryogenesis occurred via the callus tissue regenerated around the ovary as well as on the abaxial and adaxial side of ligulate florets, applying each combination of the quantitative and qualitative composition of growth regulators, both on the whole and on transversely-cut-into-half ligulate florets (tab. 1). Most embryos got regenerated on cut ligulate florets inoculated on the medium supplemented with 4.65 µM KIN as well as 18.08 µM 2,4-D. The share of the explants initiating regeneration in that case accounted for 85%.

Tanaka et al. [2000] induced somatic embryogenesis in chrysanthemum 'Aboukyu' on, horizontally inoculated with the abaxial side, fragments of ligulate florets 1 cm long. The experiment involved the use of media containing 0.46 µM KIN as well as

Table 1. Effect of the PGRs added to the MS medium and the explant type on the efficiency of somatic embryos regeneration

Type and concentration (µM) of PGRs			Ligulate floret	Number of embryos per inoculated explant	Explants regenerating embryos (%)
2,4-D	KIN	BAP			
18.08	0	0	whole	1.77^{bc}*	68.33^{a}
			cut	1.45^{bc}	65.00^{a}
18.08	4.65	0	whole	3.27^{b}	68.33^{a}
			cut	5.70^{a}	85.00^{a}
18.08	9.30	0	whole	2.80^{bc}	75.00^{a}
			cut	1.95^{bc}	60.00^{a}
18.08	23.25	0	whole	0.91^{c}	60.00^{a}
			cut	1.35^{bc}	50.00^{a}
18.08	0	4.44	whole	0.95^{c}	35.00^{a}
			cut	2.78^{bc}	63.33^{a}
18.08	0	8.88	whole	0.55^{c}	30.00^{a}
			cut	1.55^{bc}	65.00^{a}
18.08	0	22.20	whole	0.60^{c}	35.00^{a}
			cut	0.60^{c}	36.67^{a}

* a, b, c… – means in columns followed by the same letter do not differ significantly at P = 0.05 (Newman-Keuls test)

57.08 μM IAA; IBA; 2,4-D or NAA. Somatic embryogenesis was initiated by the explants inoculated onto the medium with KIN and IAA as well as with KIN and NAA. IBA and 2,4-D did not induce embryogenesis. Successive research used the media supplemented only with KIN or IAA or the combination of KIN and IAA without or with BAP at various concentrations. Embryoids did not get formed on the media not containing growth regulators or supplemented only with auxin or cytokinin. The best results were reported again on the medium with 0.46 μM KIN and 57.08 μM IAA. Adding BAP decreased the percentage share of explants regenerating embryos. In *Fritillaria imperialis* L. petals were placed onto the media supplemented with three growth regulators at various concentrations – BAP (0; 0.44; 4.44 μM), NAA (0; 1.61; 3.22 μM), IAA (0; 2.28; 4.57 μM). Non-embryogenic callus was getting formed on the explants placed on the media containing auxin only (3.22 μM NAA or 3.22 μM NAA + 2.28 μM IAA) or BAP at a high concentration and auxin (4.44 μM BAP + 1.61 μM NAA + 2.28 μM IAA). Embryogenic callus tissue was received only on the medium with BAP at a low concentration and auxin at a higher concentration (0.44 μM BAP + 3.22 μM NAA + 2.28 μM IAA) [Mohammadi-Dehcheshmeh et al. 2007]. In *Chamomilla recutita* L. the whole ligulate florets served as explants and parts of inflorescences 0.5 × 0.5 cm in size containing tubular florets [Kintzios and Michaelakis 1999]. The media were added with BAP, KIN, NAA, or 2,4-D in various quantitative and qualitative combinations. The indirect somatic embryogenesis occured only on the explants containing tubular florets, on the media with 8.88 μM BAP as well as 1.07 μM NAA or 11.5 μM KIN as well as 26.8 μM NAA added. However, only the embryoids on the medium with KIN and NAA were undergoing successive, after the globular one, development stages.

Table 2. Effect of the PGRs added to the MS medium and the explant type on the regeneration of somatic embryos in different development stages

Type and concentration (μM) of PGRs			Ligulate floret	Number of somatic embryos per inoculated explant				
				development stages				
2,4-D	KIN	BAP		globular	heart	torpedo	cotyledonary	mature
18.08	0	0	whole	0.00^{a}*	0.10^{a}	0.83^{bc}	0.62^{b}	0.22^{b}
			cut	0.05^{a}	0.00^{a}	0.80^{bc}	0.55^{b}	0.05^{b}
18.08	4.65	0	whole	0.20^{a}	0.45^{a}	1.07^{abc}	0.70^{b}	0.85^{a}
			cut	0.35^{a}	0.80^{a}	2.05^{a}	1.80^{a}	0.70^{ab}
18.08	9.30	0	whole	0.25^{a}	0.30^{a}	1.35^{ab}	0.70^{b}	0.20^{b}
			cut	0.05^{a}	0.25^{a}	0.65^{bc}	0.85^{b}	0.15^{b}
18.08	23.25	0	whole	0.07^{a}	0.10^{a}	0.22^{c}	0.30^{b}	0.22^{b}
			cut	0.05^{a}	0.10^{a}	0.80^{bc}	0.40^{b}	0.00^{b}
18.08	0	4.44	whole	0.15^{a}	0.10^{a}	0.25^{c}	0.20^{b}	0.25^{b}
			cut	0.25^{a}	0.22^{a}	1.10^{abc}	0.86^{b}	0.35^{ab}
18.08	0	8.88	whole	0.15^{a}	0.10^{a}	0.20^{c}	0.10^{b}	0.00^{b}
			cut	0.15^{a}	0.30^{a}	0.20^{c}	0.60^{b}	0.30^{b}
18.08	0	22.20	whole	0.20^{a}	0.25^{a}	0.15^{c}	0.00^{b}	0.00^{b}
			cut	0.00^{a}	0.05^{a}	0.40^{bc}	0.10^{b}	0.05^{b}

* a, b, c… – means in columns followed by the same letter do not differ significantly at P =0.05 (Newman-Keuls test)

A comparison of our results with those reported by the above researchers suggests that the process of somatic embryogenesis depends heavily on the quantitative and qualitative composition of growth regulators, however, there is also found a very clear effect of the genotype as well as the kind of the explant. In chrysanthemums somatic embryogenesis is enhanced by the media containing both auxin and cytokinin throughout the culture period, which also coincides with the report by Lema-Rumińska [2012]. Similarly Naing et al. [2013] received very good results when somatic embryogenesis was induced from *in vivo* grown leaf explants of chrysanthemum 'Euro' inoculated on MS medium supplemented with 9.04 µM 2,4-D and 9.30 µM KIN. In somatic embryogenesis in chrysanthemum auxin is usually added to media at high concentrations. Somatic embryos of good quality were regenerated when media were supplemented with cytokinin at the same time.

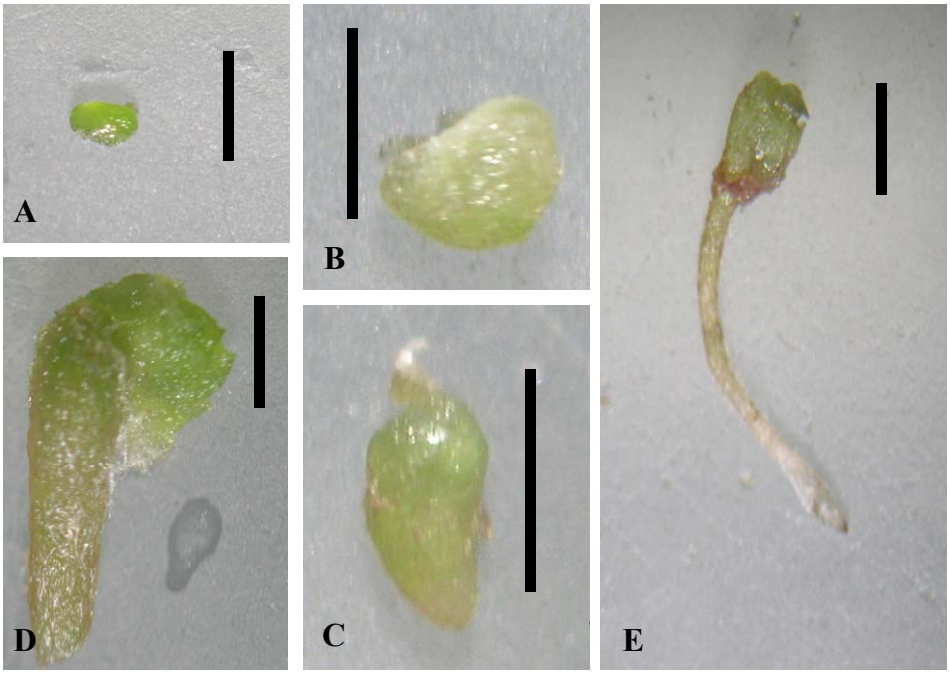

Fig. 1. Development stages of somatic embryos isolated from ligulate florets of chrysanthemum: A – globular; B – heart; C – torpedo; D – cotyledonary; E – mature; 1 bar = 1 mm

The present research did not show the relationship between the number of embryos at the globular stage and the heart stage and the concentration and the kind of growth regulators contained in the medium and the kind of the explant applied (tab. 2; fig 1). Most embryos at the torpedo stage regenerated on transversely-cut-into-half ligulate florets placed on the media supplemented with 4.65 µM KIN or 4.44 µM BAP as well as 18.08 µM 2,4-D as well as on the whole ligulate florets placed onto media with 4.65 or 9.30 µM KIN as well as 18.08 µM 2,4-D added. On the ligulate florets transversely-

cut-into-half placed onto the medium supplemented with 4.65 µM KIN as well as 18.08 µM 2,4-D there were produced most embryoids at the cotyledonary stage, while most mature embryos were observed on the whole ligulate florets placed onto the medium with 4.65 µM KIN as well as 18.08 µM 2,4-D as well as on the transversely-cut-into-half ligulate florets placed on the medium with 4.65 µM KIN or BAP as well as 18.08 µM 2,4-D. Lema-Rumińska and Niedojadło [2013] found in the medium containing only auxin 2,4-D the later stages of embryo development: torpedo, cotyledonary and mature. However, for media containing, besides 2,4-D also cytokinin, the percentage of maturity stages was significantly lower, ranging from 0 to 36%. The presence of somatic embryos at various development stages after 30 days of culture on the surface of ligulate florets of chrysanthemum 'Purima' were also noted by Mandal and Datta [2005]. According to May and Trigiano [1991], the capacity for the formation of mature embryos depends on the chrysanthemum genotype.

Table 3. Effect of the PGRs added to the MS medium and the explant type on the adventitious roots regeneration

Type and concentration (µM) of PGRs			Ligulate floret	Number of roots per inoculated explant	Explants regenerating roots (%)
2,4-D	KIN	BAP			
18.08	0	0	whole	0.37^{bc}*	11.66^{c}
			cut	0.10^{bc}	10.00^{c}
18.08	4.65	0	whole	1.40^{a}	53.33^{a}
			cut	0.25^{bc}	5.00^{c}
18.08	9.30	0	whole	0.30^{bc}	15.00^{c}
			cut	0.75^{b}	40.00^{b}
18.08	23.25	0	whole	0.20^{bc}	10.00^{c}
			cut	0.00^{c}	0.00^{c}
18.08	0	4.44	whole	0.10^{bc}	10.00^{c}
			cut	0.20^{bc}	5.00^{c}
18.08	0	8.88	whole	0.00^{c}	0.00^{c}
			cut	0.30^{bc}	15.00^{c}
18.08	0	22.20	whole	0.10^{bc}	10.00^{c}
			cut	0.00^{c}	0.00^{c}

*a, b, c… – means in columns followed by the same letter do not differ significantly at P = 0.05 (Newman-Keuls test)

Most adventitious roots got regenerated on the whole ligulate florets placed onto the medium supplemented with 4.65 µM KIN as well as 18.08 µM 2,4-D. In that experimental combination the capacity for initiating rhizogenesis was reported in more than 53% explants (tab. 3). The regeneration of adventitious roots during the proces of somatic embryogenesis is not unexpected and was also observed by many other authors.

Bhattacharya et al. [1990] on the medium with 4.52 or 9.04 μM 2,4-D observed an intensive direct regeneration of roots from the fragments of laminas in 'Birbal Sahni' chrysanthemum. Tanaka et al. [2000] obtained adventitious roots regeneration from the fragments of ligulate florets in chrysanthemum 'Aboukyu' placed on the medium with 0.46 μM KIN as well as 57.08 μM IAA; IBA; 2,4-D or NAA. It is common knowledge that a high concentration of auxin and average/low of cytokinin enhance the root regeneration [Ilzuka et al. 1973]. However, in the present research the capacity for rhizogenesis was also demonstrated by whole ligulate florets placed on the media with a higher concentration of cytokinin than auxin. The effect can be due to the genotype differences in the capacity for uptaking from the medium and then metabolizing respective growth regulators [Nahid et al. 2007].

CONCLUSIONS

1. The somatic embryogenesis occurs on the ligulate florets inoculated onto the MS medium with 18.08 μM 2,4-D as well as with this auxin and 4.44; 8.88; 22.20 μM BAP or 4.65; 9.30; 23.25 μM KIN.

2. Most embryos regenerate on transversely-cut-into-half ligulate florets inoculated onto the medium with 4.65 μM KIN and 18.08 μM 2,4-D.

REFERENCES

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

Broertjes C., van Harten A.M., 1988. Applied mutation breeding for vegetatively propagated crops. Elsevier, Amsterdam, 29–59.

Chakrabarty D., Datta S.K., 2010. Management of chimera and *in vitro* mutagenesis for development of new flower colour/shape and chlorophyll variegated mutants in chrysanthemum. In: Floriculture. Role of tissue culture and molecular techniques, Datta S.K., Chakrabarty D. (eds.). Pointer Publishers, Jaipur, 157–164.

Chakrabarty D., Mandal A.K.A., Datta S.K., 1999. Management of chimera through direct shoot regeneration from florets of chrysanthemum (*Chrysanthemum morifolium* Ramat.). J. Hort. Sci. Biotechnol., 74(3), 293–296.

Dodeman V.L., Ducreux G., Kreis M., 1997. Zygotic embryogenesis versus somatic embryogenesis. J. Exp. Bot., 48(313), 1493–1509.

Gahan P.B., George E.F., 2008. Adventitious regeneration. In: Plant propagation by tissue culture 3rd edition. Volume 1. The background, George E.F., Hall M.A., de Klerk G.-J. (eds). Springer, Dordrecht, 358–389.

Ilzuka M., Matsumoto E., Doi A., Madrigal R., Fukushima A., 1973. Tubular floret culture of chrysanthemum and cineraria *in vitro*. Jpn. J. Gen., 48, 2, 79–87.

Kintzios S., Michaelakis A., 1999. Induction of somatic embryogenesis and *in vitro* flowering from inflorescences of chamomile (*Chamomilla recutita* L.). Plant Cell Rep., 18, 684–690.

Lema-Rumińska J., 2012. Somatic embryogenesis as a chrysanthemum propagation tool. 2nd Symposium on Horticulture in Europe, July 1–5, Angers, France, 3, 188.

Lema-Rumińska J., Niedojadło J., 2013. Developmental stages of somatic embryos in chrysanthemum depending on the cultivar and the growth regulators concentration in the medium. 8th International Symposium on *In Vitro* Culture and Horticultural Breeding, Coimbra, Portugal. Book of Abstracts P76, 164.

Lema-Rumińska J., Śliwińska E., 2009. Ocena stabilności roślin uzyskanych z zarodków somatycznych u chryzantemy wielkokwiatowej (*Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. Zesz. Probl. Post. Nauk Roln., 539(2), 425–432.

Malaure R.S., Barclay G., Power J.B., Davey M.R., 1991. The production of novel plants from florets of *Chrysanthemum morifolium* using tissue culture 2. Securing natural mutations (sports). J. Plant Physiol., 139, 14–18.

Mandal A.K.A., Datta S.K., 2005. Direct somatic embryogenesis and plant regeneration from ray florets of chrysanthemum. Biol. Plant., 49(1), 29–33.

May R.A., Trigiano R.N., 1991. Somatic embryogenesis and plant regeneration from leaves of *Dendranthema grandiflora*. J. Amer. Soc. Hort. Sci., 116(2), 366–371.

Mohammadi-Dehcheshmeh M., Khalighi A., Naderi R., Ebrahimie E., Sardari M., 2007. Indirect somatic embryogenesis from petal explant of endangered wild population of *Fritillaria imperialis*. Pak. J. Biol. Sci., 10(11), 1875–1879.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15, 473–497.

Naing A.H., Kim C.K., Yun B.J., Jin J.Y., Lim K.B., 2013. Primary and secondary somatic embryogenesis in *Chrysanthemum* cv. Euro. Plant Cell Tiss. Org. Cult., 112, 361–368.

Nahid J.S., Shyamali S., Kazumi H., 2007. High frequency shoot regeneration from petal explants of *Chrysanthemum morifolium* Ramat. *in vitro*. Pak. J. Biol. Sci., 10(19), 3356–3361.

Pavingerová D., Dostál J., Bísková R., Benetka V., 1994. Somatic embryogenesis and *Agrobacterium*-mediated transformation of chrysanthemum. Plant Sci., 97, 95–101.

Stewart R.N., Dermen H., 1970. Somatic genetic analysis of the apical layers of chimeral sports in chrysanthemum by experimental production of adventitious shoots. Am. J. Bot., 57(9), 1061–1071.

Tanaka K., Kanno Y., Kudo S., Suzuki M., 2000. Somatic embryogenesis and plant regeneration in chrysanthemum (*Dendranthema grandiflorum* (Ramat.) Kitamura). Plant Cell Rep., 19, 946–953.

Tymoszuk A., Zalewska M., 2014. Biological factors affecting regeneration of adveytitious shoots from *in vitro* isolated ligulate florets of chrysanthemum. Acta Sci. Pol., Hortorum Cultus 13(3), 155–165.

Zalewska M., Lema-Rumińska J., Miler N., 2007. *In vitro* propagation using adventitious buds technique as a source of new variability in chrysanthemum. Sci. Hortic. 113, 70–73.

REGENERACJA ZARODKÓW SOMATYCZNYCH Z IZOLOWANYCH *in vitro* KWIATÓW JĘZYCZKOWATYCH CHRYZANTEMY

Streszczenie. Wiele mutantów chryzantem jest chimerami zbudowanymi z tkanek o różnym składzie genetycznym. Regeneracja *in vitro* zarodków somatycznych z całych objętych mutacją kwiatów języczkowatych lub tylko z ich niewielkich zmutowanych fragmentów może doprowadzić do separacji komponentów składowych chimery i w rezultacie do uzyskania nowej, oryginalnej odmiany. W badaniach określono wpływ regulatorów wzrostu (2,4-D; KIN lub BAP) oraz rodzaju eksplantatu (cały lub przecięty poprzecznie na pół

kwiat języczkowaty) na wydajność embriogenezy somatycznej u *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam. 'Cool Time'. Do indukcji embriogenezy somatycznej oraz do regeneracji zarodków somatycznych zastosowano pożywkę MS [Murashige and Skoog 1962] uzupełnioną jedynie 18,08 µM 2,4-D, a także tą auksyną oraz 4,44; 8,88; 22,20 µM BAP lub 4,65; 9,30; 23,25 µM KIN. Najlepsze rezultaty uzyskano, inokulując przecięte poprzecznie na pół kwiaty języczkowate na pożywkę z 4,65 µM KIN i 18,08 µM 2,4-D. Uzyskane wyniki mogą przyczynić się do zwiększenia prawdopodobieństwa separacji komponentów chimer w hodowli chryzantem.

Słowa kluczowe: *Chrysanthemum* × *grandiflorum* (Ramat.) Kitam., embriogeneza somatyczna, regulatory wzrostu

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