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MICROPROPAGATION OF WILD CHICORY (*Cichorium intybus* L. var. *silvestre* Bisch.) FROM LEAF EXPLANTS

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Abstract. *Cichorium intybus* is edible, medicinal and forage plant. The pharmaceutical raw materials were obtained from wild chicory (var. *silvestre*). Currently, farmers are increasingly assume plantations of wild chicory, and breeders are attempting to produce cultivars for medicinal purposes. In the modern breeding of chicory important feature is the ability to clonal propagation *in vitro* culture. The aim of our study was to assess capacity of natural population of wild chicory for plant regeneration from leaf explants. In the first was examined the effect of 16 combinations of various concentrations of IAA and 2iP on the regeneration of shoots from leaf explants (0.5 cm^2). After that, 25 plants were propagated on the medium which was found as optimal. Then, their callus growth and shoots regeneration capacities were compared. Later on, was examined the effect of various IAA concentrations on the rooting of shoots. The majority of the shoots was regenerated from callus but direct organogenesis was also observed (8%). Shoot regeneration was found to be the most efficient on MS medium containing 0.5 mg dm^{-3} IAA and 4 mg dm^{-3} 2iP – 97% of the explants produced shoots, while the average number of shoots was 15.5. The amount of callus was found to be a highly heritable trait ($h^2 = 0.83$). Lower the heritability coefficients were obtained for the number of shoots per explant (0.55) and the average shoot weight (0.40). The wild chicory shoots rooted easily. The number and weight of roots increased with the increasing concentration of IAA.

Key words: *Cichorium intybus*, *in vitro* propagation, organogenesis, heritability coefficients

INTRODUCTION

Chicory (*Cichorium intybus* L.) is a member of the *Asteraceae* family. According to Vavilov [1951] the species originates from the Mediterranean region. In the historical times, wild chicory and chicory cultivars were popularised across Europe, Africa, Asia,

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the Americas, Australia and oceanic islands [Rickett 1967, Bais and Ravishankar 2001, Tutin at al. 2010]. The *C. intybus* species is characterised by high diversity with various botanical varieties distinguished. In Poland, wild chicory – *C. intybus* L. var. *silvestre* Bisch. is found in natural localities and there are also two cultivated forms: salad chicory – *C. intybus* L. var. *foliosum* Bisch. and root chicory – *C. intybus* L. var. *sativum* Bisch. Wild chicory is a perennial plant up to 120 cm tall with a long tap root with very few branches. The cultivated forms are biennial plants which differ in the proportions of biomass accumulated in leaves and roots, and are grown for different purposes. Salad chicory is eaten as a vegetable. The main use of root chicory is the production of coffee substitutes and food concentrates from dried roots [Bais and Ravishankar 2001, Senderski 2009]. Both cultivars are also grown as a forage crop [Li and Kemp 2005].

Chicory is an important medicinal plant which accumulates various specific organic compounds, such as storage polysaccharide inulin, sesquiterpene lactones, cumarins, phenolic acids and flavonoids [Bais and Ravishankar 2001, Senderski 2009]. Medicinal substances are found in all of its botanical varieties, but it is *C. intybus* var. *silvestre* which is most often used for this purpose. It produces less biomass but the concentration of secondary metabolites in this variety is much higher than in the remaining ones. In Poland, the demand for chicory raw materials is covered by plants growing in natural locations and by field cultivation.

In folk medicine, infusions of chicory roots and herb are used to treat gastrointestinal diseases, hepatic cirrhosis and spleen swelling; they are also used externally in eczema treatment. The official medicine uses chicory preparations and herbal mixtures, which are most frequently used as support treatment along with synthetic drugs. Chicory preparations regulate the metabolism, improve the digestion and absorption of food, have a diuretic effect and calm the nervous system [Bais and Ravishankar 2001, Senderski 2009].

Chicory cultivars and separate plants in wild chicory populations differ in terms of morphology and the content of secondary metabolites, which makes it possible to conduct selection to obtain forms with desired properties. The chemical composition of the biomass can also be altered by the production of transgenic forms [Genga et al. 1994]. A rapid propagation of selected genotypes and genetically modified forms of chicory can be carried out by means of *in vitro* culturing. Chicory can be multiplied *in vitro* by somatic embryogenesis [Bellettre et al. 1999], by direct organogenesis from the shoot apices [Previati et al. 2005] and by the regeneration of adventitious shoots [Rehman et al. 2003]. Much better results were obtained when the plants were propagated through adventitious shoots.

Studies on the potential for micropropagation through adventitious shoots were performed on wild chicory (var. *silvestre*) [Rehman et al. 2003, Yucesan et al. 2007] and cultivated forms (var. *sativum* and var. *foliosum)* [Pieron et al. 1993, Nandagopal and Ranjitha Kumari 2006]. In all studies, regeneration of shoots was preceded by the development of callus. Efficiency of callus induction and shoot regeneration depend on the type of explants and growth regulators (especially the ratio between auxin and cytokinin). The best results were obtained using leaf explants, callus weaker growth and fewer shoots were obtained from hypocotyles, petioles and roots [Velayutham et al. 2006, Yucesan et al. 2007]. Research Rehman et al. [2003] shown that the efficiency of the

regeneration of shoots in the chicory can be increased by means of casein hydrolyzate (CH). The study Nandagopal and Ranjitha Kumari [2006] observed a beneficial effect of adenine sulphate (ADS). Young shoots of chicory produced roots on essential media without hormones, but the results of rooting were better in auxin application.

In the literature on chicory lack of information on the inheritance of the ability to micropropagation. Tests were performed on a single individual cultivars and wild chicory populations. No attempt cloning of various materials *C. intybus* (cultivars, wild populations or individual plants) under the same conditions. We did not find information about it attempts to estimate heritability, and studies aimed at identifying molecular markers of DNA coupled with the ability to micropropagation.

The aim of our study was to assess the capacity of natural populations of wild chicory for plant regeneration from leaf blade fragments. An attempt was made at finding the optimal combination of isopentenyladenine (2iP) cytokinin and indoleacetic acid (IAA) auxin. Finally, the heritability of the following traits was assessed: the amount of callus, the number of shoots per explant and the average shoot weight.

MATERIAL AND METHODS

In late autumn 2011, several dozens of wild chicory shoot apices (20–25 cm) were gathered from the area of uncultivated land (about 150 ha). The parent plants were choose from these which grown longways the track, in distances 20–30 m. The shoots were threshed manually; the seeds were mixed with wet sand and placed in a refrigerator at 3–4°C. Two weeks later, they were sown in groups of ten into twelve 3 dm^3 pots filled with gardening soil. The onset of germination was observed after 10 days. After two weeks, the seedlings were thinned and three plants were left in each pot. The research began in another three weeks. At this time, all plants were in the rosette stage. They differed in terms of: the number, size and shape of the leaves.

The first experiment examined the effect of various concentrations of 2iP cytokinin and IAA auxin on the induction of callus and shoot regeneration. Three fully developed leaves were harvested from each of the 30 plants. Fragments with an area of 2–3 cm^2 were cut off from leaf blades. Each fragment was assigned to one experimental set, disinfected for 7 minutes in 0.1% $HgCl_2$ and rinsed in sterile water. Three explants with an area of 0.5 cm^2 were obtained from each leaf fragment and plated on agar-solidified media. All the media contained the basic components of MS medium [Murashige and Skoog 1962], the difference being the concentrations of 2iP (0.5–4 mg dm^{-3}) and IAA (0.05–0.4 mg dm^{-3}). The explants were placed in Petri dishes of 9 cm diameter, six into each dish, while each experimental set consisted of 15 dishes. The dishes were covered with plastic wrap and placed in a growth chamber with a temperature of 25°C, photoperiod of 12 h and white light with an intensity of 25–30 μmol s^{-1} m^{-2}. After 28 days, the percentages of explants with developed shoots and these with roots were calculated for each medium. Forty totipotent explants were rated in a 9° scale (see tab. 1) for the amount of callus. The number and weight of shoots developed by those explants, and percentage of explants with vitrified shoots were also determined.

In the second experiment, leaf explants from 25 plants were compared for their ability to develop shoots. We have reduced the number of genotypes evaluated because some plants have started the development of generative. They produced a much smaller leaves and stems, which reduced the possibility of obtaining an adequate number of explants (15 × 6 = 90). The explants were disinfected by the same method as previously and put into dishes with a medium containing 0.05 mg dm^{-3} IAA and 4 mg dm^{-3} 2iP. The dishes were then placed in a growth chamber. Four weeks later, explants which developed shoots were counted for each genotype. Forty explants were rated for the same parameters as in the first experiment.

Some of the shoots were transferred to 0.4 dm^{-3} jars with five types of rooting medium. The media contained the basic components of MS medium and differed by the concentration of IAA (0–2 mg dm^{-3}). Each experimental set consisted of 15 jars, each containing 25 ml of medium and 4 shoots. The jars were placed in a growth chamber. Three weeks later, 5 × 40 plants were examined for the following parameters: shoot weight, the number and weight of the roots and the length of the largest root. Ten plants from each type of medium were planted in 0.3 dm^{-3} pots with a sterile mixture of garden soil, perlite and sand (3:1:1). The pots were covered with plastic cups and placed in a greenhouse; the covers were removed after two weeks.

Statistical analysis of the results was performed. The significance of differences between the mean values of individual parameters was established using Tukey's method. The broad-sense heritability coefficients were calculated for the number of shoots per explant, the average shoot weight and the amount of callus, using the sum of squared deviations for each plant and for the whole experiment [Falconer 1989].

RESULTS AND DISCUSSION

In our research, the begining of callus production by the explants on all sixteen media was observed after 5–6 days (fig. 1A). The initial of shoot development was observed within 2 first weeks of the experiment. After four weeks from the establishment of the cultures, the percentage of explants which developed shoots counted for individual experimental sets ranged from 26.9 to 99.3% (tab. 1). Regardless of the concentration of IAA, the percentage of explants with shoots increased with the increasing concentration of 2iP. In about 92% of the totipotent explants, the development of buds and shoots was preceded by callus formation (fig. 1B). The remaining explants developed shoots with no previous callus formation (fig. 1C). The amount of callus depended on the composition of the medium. After four weeks, the mean ratings of the amount of callus ranged from 1.1–8.0°. The most intense callus development was observed in the explants grown on the medium containing 0.4 mg dm^{-3} IAA and 4 mg dm^{-3} 2iP. In the final phase of the shoot regeneration process, some of the explants from each medium were observed to develop roots. The percentage of explants with roots depended on the concentration of 2iP and the proportion between cytokinin and auxin. The fewest explants (2.7–6.2%) produced roots on the media with the highest concentrations of 2iP. A large percentage of explants with roots (33.7–42.5%) was found on the media containing 0.4 mg dm^{-3} IAA and 0.05 to 2 mg dm^{-3} 2iP. The growth regulators also had a signifi-

cant impact on the number of shoots produced. The average number of shoots per explant ranged from 4.2 to 15.47 in the whole experiment. At all concentrations of IAA, the number of shoots increased with the increasing concentration of 2iP. In the explants which produced more shoots, the average shoot weight was most often smaller. During the evaluation of the shoots, vitrification was observed (tab.1). It was found mainly on the leaves which were in direct contact with the medium. The percentage of explants with vitrified shoots increased with the increasing concentration of cytokinin. The highest percentage (15.5%) was observed on the medium containing 0.2 mg dm^{-3} IAA and 4 mg dm^{-3} 2iP.

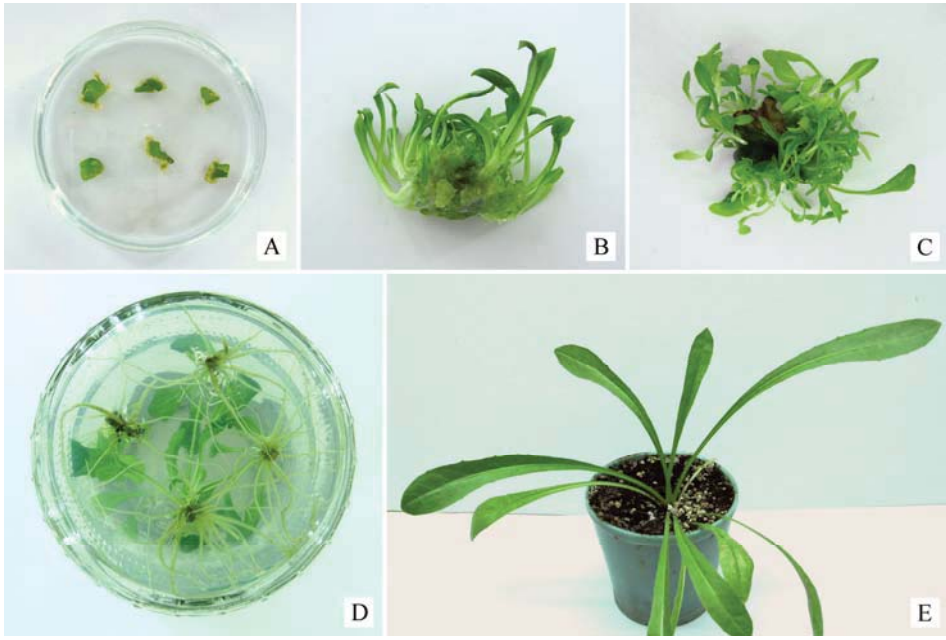

Fig. 1. Micropropagation of wild chicory: A – first steps of callus development, B – shoots regeneration from callus, C – direct shoots regeneration on leaf explant, D – roots development, E – hardened chicory plant

In the earlier studies, the efficiency of shoot regeneration from leaf explants of *C. intybus* varied. It was depended mainly on the hormonal composition of the media and the material used for the experiments. Rehman et al. [2003] were plated wild chicory leaf explants on MS medium supplemented with various concentrations of IAA (0.09–0.35 mg dm^{-3}) in combination with fixed concentrations of 6-benzylaminopurine (BAP – 1.13 mg dm^{-3}), kinetin (KIN – 1.08 mg dm^{-3}) and CH (1000 mg dm^{-3}). The formation of callus was observed after 2 weeks, shoot buds initiated growth in 30-day-old callus. The shoots were separated from the 5-week-old callus. The percentage of explants forming callus ranged from 28.8 to 84.4% in individual experimental sets.

Shoots were developed by 24.0 to 84.4% of the explants with callus, while the average number of shoots ranged from 1.26 to 5.92. Better results were obtained on the media containing KIN rather than BAP. The percentage of explants with callus and the remaining parameters were positively affected by the presence of CH. In the research by Velayutham et al. [2006], the explants were small fragments (10–15 mm) leaves. Callus was induced on medium containing 0.45 mg dm^{-3} BAP in combinations with various concentrations of auxins. The amount of callus, assessed after 30 days of culture, depended on the type of auxin. The auxins were ordered in terms of this parameter in the following way: naphtaleneacetic acid (NAA) > indolebutiric acid (IBA) > IAA > 2,4-dichlorophenoxyacetic acid (2.4-D). Shoots were regenerated from calli on medium supplemented with various concentrations of BAP, IAA and KIN. The development of shoots was more efficient on the media with BAP rather than these with KIN. In individual experimental sets, the shoots were developed from 63.3–100% the explants with callus, with the average number of shoots ranging from 2.0 to 42.92. The best results were obtained on the medium containing 0.9 mg dm^{-3} BAP and 0.175 mg dm^{-3} IAA.

Table 1. Effect of growth regulators on callus induction and shoot regeneration

Medium	Growth regulators (mg dm^{-3})		Explants with shoots (%)	Callus amount (1–9°)*	Explants with roots (%)	No. of shoots per explant	Shoot weight (mg)	Vitrification (%)
	IAA	2iP						
1.	0.05	0.5	59.7	2.10	15.7	6.43	25.27	0.5
2.	0.05	1	45.3	1.93	22.7	6.17	16.38	1.0
3.	0.05	2	85.0	1.10	18.7	10.63	18.59	3.5
4.	0.05	4	97.3	2.34	2.7	15.47	16.46	5.0
5.	0.1	0.5	26.9	1.20	17.9	4.20	19.26	0
6.	0.1	1	80.1	2.53	15.9	8.26	31.03	2.5
7.	0.1	2	70.0	1.80	13.7	9.90	19.09	7.5
8.	0.1	4	91.2	8.00	6.2	8.97	18.67	10.5
9.	0.2	0.5	43.7	2.07	20.0	5.13	20.52	3.7
10.	0.2	1	42.5	3.00	25.0	6.57	18.33	11.4
11.	0.2	2	86.2	6.80	21.2	11.20	21.09	12.5
12.	0.2	4	95.2	4.23	6.1	11.50	19.05	15.5
13.	0.4	0.5	44.4	1.93	39.5	4.03	20.87	0
14.	0.4	1	63.7	3.70	33.7	9.47	18.82	0.5
15.	0.4	2	81.2	2.67	42.5	12.87	19.14	3.2
16.	0.4	4	99.3	4.57	4.8	12.83	17.49	9.4
Mean			69.5	3.18	19.1	8.97	20.00	5.4
$LSD_{\alpha = 0.05}$			–	0.70	–	2.45	4.86	–

(1–9°)* – in scale, where; 1° – very feeble callusing, 9° – very intense callusing

Nandagopal and Ranjitha Kumari [2006] were plated chicory leaf explants on medium MS+B5, supplemented with various concentrations of IAA, KIN, BAP and ADS. The development of callus was observed after 10–15 days, while the regeneration of shoots began when the callus was 30-day-old. After 45 days of culturing, the researchers found out that ADS has a positive effect on the percentage of explants which form callus, the percentage of callus which develop shoots and the number of shoots. The best results were obtained on medium containing 1.5 mg dm^{-3} BAP, 0.5 mg dm^{-3} IAA and 0.25 mg dm^{-3} ADS. Callus was produced by 94.3% of the explants, shoots were regenerated by 93.0% of the calli, while the average callus produced 33 shoots. In the study by Yucesan et al. [2007], fragments of leaf blades have a little area (0.25 cm^2). On the medium with auxins but without cytokinins, callus appeared but no shoots were formed. The development of callus along with the regeneration of shoots was observed on the media with cytokinins (BAP, KIN and thidiazuron – TDZ) no auxins, and on the media with combinations of both hormones. Shoot development began after 9 days culturing and the effects of shoot regeneration was assessed after 4 weeks. The best results were obtained on the medium containing 0.01 mg dm^{-3} TDZ and 1 mg dm^{-3} IAA, where 100% of explants produced shoots and the average shoot number was 35.8. Among the combinations of KIN and BAP cytokinins with IAA and NAA auxins, the best results were obtained on the medium containing 0.3 mg dm^{-3} IAA and 0.5 mg dm^{-3} KIN (shoot regeneration: 100%, average number of shoots: 19.7). Vitrification of shoots was observed and it occurred more frequently on the media with NAA than on these with IAA.

In the present research, 25 wild chicory plants were compared for their micropropagation ability. The medium which was considered optimal was used. It contained 0.04 mg dm^{-3} IAA and 4 mg dm^{-3} 2iP. The plants differed in their ability to produce callus and regenerate shoots (tab. 2). In 19 plants, all leaf explants produced shoots, while in the remaining genotypes the percentage of explants with shoots ranged from 73.3 to 93.3%. There were significant differences in the amount of callus, the average score ranging from 1.33 to 8.33°. The average number of shoots per one totipotent explant ranged from 2.20 to 22.78, while the average weight of shoot ranged from 10.68 to 36.63 mg. The explants from 12 plants did not form roots, while the percentage of explants with roots in the remaining 13 genotypes ranged from 10 to 58.3%. The highest broad-sense heritability coefficient was obtained for the amount of callus ($h^2 = 0.83$); the coefficients for the number of shoots per explant (0.55) and the average weight of shoot (0.40) were lower.

Within three weeks, all chicory shoots plated onto the rooting media developed roots (fig. 1D). On the medium without auxin, roots developed without the growth of callus. At low concentrations of IAA, small amount of callus was produced and it did not have any significant impact on the rhizogenesis. At 2 mg dm^{-3} IAA, more callus grew at the base of the shoots, which delayed the development of roots. The weight of the plants grown on the control medium was lower that of these grown on the media containing various concentrations of IAA. However, no significant differences were observed in terms of the following parameters: the weight of shoots, length of stems and number of leaves (tab. 3). The exogenous auxin had a strong impact on the development of root systems. The plants grown on the medium without IAA produced fewer roots but the

roots were longer than these developed in the media with auxin. The number and weight of roots as well as the percentage share of root weight in the weight of the whole plant rised with the increasing concentration of IAA (tab. 3). The best plants were obtained on the medium containing 2 mg dm^{-3} IAA. They were characterised by a high total weight as well as a large number and high weight of roots. The weakest plants were obtained on the medium without IAA. These plants were characterized by a relatively high total weight but a small number and low weight of roots.

Table 2. Chicory plants rating for its ability to regenerate shoots

Plant	Explants with shoots (%)	Explants with roots (%)	No. of shoots per explant	Shoot weight (mg)	Callus amount (1–9°)*
1.	100	0	22.78	29.03	3.00
2.	100	0	17.27	24.17	2.13
3.	100	16.7	13.63	16.28	7.77
4.	100	0	13.17	38.71	1.33
5.	100	0	13.03	24.06	2.80
6.	100	27.8	12.70	18.86	4.70
7.	100	47.2	12.53	26.12	4.77
8.	100	11.1	11.93	27.21	7.03
9.	100	55.5	11.80	24.86	7.60
10.	100	0	9.80	23.64	4.70
11.	100	58.3	9.30	23.86	3.63
12.	100	38.9	8.50	19.35	5.70
13.	100	0	8.37	26.93	4.37
14.	100	0	7.90	27.33	3.87
15.	100	16.7	7.07	21.93	5.10
16.	100	0	6.70	22.59	4.07
17.	100	0	5.67	14.09	8.13
18.	100	0	5.50	10.68	4.80
19.	100	0	3.90	28.52	8.33
20.	97.2	16.7	11.53	26.30	2.07
21.	93.3	46.7	7.40	36.63	3.73
22.	90.0	0	5.17	15.36	8.80
23.	83.3	38.9	9.23	23.18	3.97
24.	76.7	30.0	5.23	17.75	6.80
25.	73.3	10.0	2.20	20.40	1.97
Mean	96.5	16.6	9.69	23.51	4.88
$LSD_{\alpha = 0.05}$	–	–	2.11	3.91	0.49
h^2	–	–	0.54	0.40	0.83

(1–9°)* – in scale (see tab. 1), h^2 – the heritability coefficient

Table 3. Rooting phase of shoots

	Medium	Plant weight (mg)	Shoot weight (mg)	Roots weight (mg)	Share of roots in plant weight (%)	Stem length (cm)	Nodes number	Leaves number	Roots number	Root length (cm)
1.	control	270.0	258.2	11.8	4.4	2.29	1.08	6.77	2.03	4.52
2.	0.25 mg dm^{-3} IAA	355.5	317.6	37.9	10.8	2.28	1.67	6.30	2.67	3.23
3.	0.5 mg dm^{-3} IAA	391.6	313.0	78.6	20.1	3.31	2.00	6.43	5.53	3.63
4.	1.0 mg dm^{-3} IAA	360.3	256.2	104.1	29.0	2.49	1.83	6.33	5.00	3.62
5.	2.0 mg dm^{-3} IAA	390.4	251.6	138.8	35.6	3.05	2.13	6.73	7.10	3.41
Mean		353.6	279.3	74.2	20.0	2.68	1.74	6.51	4.47	3.68
$LSD_{\alpha = 0.05}$		61.5	ns	28.6	-	ns	0.50	ns	1.28	ns

The high root development capacity of chicory shoots regenerated *in vitro* was observed in many studies. In the research by Rehman et al. [2003], 100% of shoots regenerated from leaf explants on MS medium supplemented with 0.041 mg dm^{-3} IBA rooted within two weeks. Nandagopal and Ranjitha Kumari [2006] observed the rooting of chicory shoots planted on MS+B5 medium supplemented with various auxins. The highest percentage of rooted shoots was observed for three auxins used at the concentration of 1 mg dm^{-3} (IBA – 100%, IAA – 92.3% and NAA – 78.6%). The highest average length of roots was obtained on the medium with IBA, while it was lower on these with IAA and NAA. In the research by Velayutham et al. [2006], IBA auxin also gave better results than IAA and NAA. Within 30 days, 96.6% of the shoots plated on MS medium containing 2.03 mg dm^{-3} IBA developed roots. The highest root length was obtained at the lowest concentrations of auxins. At high concentrations of IBA, thick main roots were developed along with lateral ones similar to hairy roots. No secondary roots were developed on the media with IAA and NAA. In the research conducted by Yucesan et al. [2007], the chicory shoots rooted better on MS medium supplemented with IAA rather than IBA. On the medium containing 0.5 mg dm^{-3} IAA, 100% of shoots developed roots, while the average number of roots was 4.2. For IBA, the most effective rhizogenesis (68%) was observed at the concentration of 1 mg dm^{-3}. When the concentrations of auxins were increased to 2 mg dm^{-3}, callus was developed. IBA induced a more intense callus growth than IAA.

In the present study, the wild chicory plants did not wither after they were transplanted to sterile soil mixture and were not significantly affected by the gradual reduction in air humidity or temperature fluctuations. After 28 days from the transfer to a greenhouse with natural light and variable temperature, all the plants were still alive and developed normally (fig. 1E).

In the research conducted by other authors, the effects of acclimatization of chicory plants regenerated *in vitro* depended mainly on the methodology used. Rehman et al. [2003] planted the rooted plants to sterile sand or soil mixture supplemented with half-strength MS salts and kept them for 2 weeks in saturated humidity conditions. Then, the plants were transplanted into pots with soil and growth at 30°C under natural light con-

ditions. 56% of the plants survived the hardening. In the Velayutham et al. [2006] study, chicory plants were washed with sterile water and planted to pots containing sterile gardening soil. The pots were covered with porous polyethylene bags and maintained in the culture room. After 2 weeks, the covers were removed and the pots were transferred to net-house. 100% of the plants survived the acclimatization. In the research by Yucesan et al. [2007], 85% of the plants survived the hardening. The plants were washed from the medium, planted in pots with sterile compost and kept for 2 weeks in a growth chamber with artificial light, fixed temperature and high air humidity. Then, they were transferred to a room with variable temperature and lower humidity.

CONCLUSIONS

1. The wild chicory shoots were regenerated mainly with callus formation, but about 8% of the explants developed shoots with no previous callus growth.

2. The efficiency of shoot regeneration depended on the hormone concentrations and the proportion between 2iP and IAA. The best results were achieved on the medium containing 0.05 mg dm^{-3} of IAA and 4 mg dm^{-3} of 2iP.

3. On the many of the media, a number of leaf explants began to develop roots towards the end of the shoots regeneration process. The percentage of explants with roots depended on the concentration of cytokinin and the proportions between IAA and 2iP.

4. The research has shown that selection can be carried out in wild chicory population for the ability to regenerate shoots from leaf explants. On the medium which considered optimal the examined plants were significantly differed in every compared features. The highest broad-sense heritability coefficient was obtained for the amount of callus ($h^2 = 0.83$). The coefficients for the number of shoots per explant and the average weight were lower.

5. The wild chicory shoots rooted easily. The number and weight of roots as well as the percentage share of root weight in the total weight of the plant increased with the increasing concentration of IAA.

6. A favourable property of the plants obtained was their ability for rapid acclimatization.

REFERENCES

Bais H.P., Ravishankar G.A., 2001. *Cichorium intybus* L. – cultivation, processing, utility, value addition and biotechnology, with an emphasis on current status and future prospects. J. Sci. Food Agric. 18, 467–484.

Bellettre A., Couillerot J.P., Vasseur J., 1999. Effect of glycerol on somatic embryogenesis in *Cichorium* leaves. Plant Cell. Rep. 19, 26–31.

Falconer D.S., 1989. Introduction to quantitative genetics. 3rd Ed. Longman Scientific and Technical, Harlow.

Genga A., Giansante L., Bernacchia G., Allavena A., 1994. Plant regeneration from *Cichorium intybus* L. leaf explants transformed by *Agrobacterium tumefaciens*. J. Genet. Breed. 48, 25–32.

Li G.D., Kemp P.D., 2005. Forage chicory (*Cichorium intybus* L.): A review of its agronomy and animal production. Adv. Agron. 88, 187–222.

Nandagopal S., Ranjitha Kumari B.D.R., 2006. Adenine sulphate induced high frequency shot organogenesis in callus and *in vitro* flowering of *Cichorium intybus* L. cv. Fokus – a potent medicinal plant. Acta Agricult. Slov. 87 (2), 415–425.

Pieron S., Belaizi M., Boxus P., 1993. Nodule culture, a possible morphogenetic pathway in *Cichorium intybus* L. propagation. Sci. Hort. 53, 1–11.

Previati A., Benelli C., Da Re F., De Carlo A., Vettori C., Lambardi M., 2005. *In vitro* propagation and conserwation of red chicory germplasm. The role of biotechnology. Villa Gualiano, Turin, Italy, 5–7 March, 207–208.

Rehman R.U., Israr M., Srivastava P.S., Bansal K.C., Abdin M.Z., 2003. *In vitro* regeneration of witloof chicory (*Cichorium intybus* L.) from leaf explants and accumulation of esculin. In Vitro Cell. Dev. Biol. – Plant 39, 142–146.

Rickett H.W., 1967. Wild flores of the United States. Parts 1–4. New York, McGraw-Hill Book Co.

Senderski M.E., 2009. Praktyczny poradnik o ziołach i ziołolecznictwie. Wyd. K.E. Liber, Warszawa, 102–103.

Tutin T.G., Burges N.A., Chater A.O., Edmondson J.R., Heywood V.H., Moore D.M., Valentine D.H., Walters S.M., Webb D.A., 2010. Flora Europea. Cambridge University Press. Vol. 4, 304–305.

Velayutham P., Ranjithakumari B.D., Baskaran P., 2006. An efficient *in vitro* plant regeneration system for *Cichorium intybus* L. – an important medicinal plant. J. Agric. Tech. 2(2), 287–298.

Vavilov N.L., 1951. The origin, variation, immunity and breeding of cultivated plants. Chron. Bot. 13, 1–366.

Yucesan B., Turker A.U., Gurel E., 2007. TDZ-induced high frequency plant regeneration through multiple shoot formation in witloof chicory (*Cichorium intybus* L.). Plant Cell Tiss. Organ Cult. 91, 243–250.

MIKROROZMNAŻANIE CYKORII PODRÓŻNIK (*Cichorium intybus* L. var. *silvestre* Bisch.) Z EKSPLANTATÓW LIŚCIOWYCH

Streszczenie. *Cichorium intybus* jest rośliną jadalną, leczniczą i pastewną. Surowce lecznicze były do niedawna uzyskiwane z roślin cykorii dzikiej (var. *silvestre*). Obecnie rolnicy coraz częściej zakładają plantacje „dzikiej cykorii", a hodowcy podejmują próby wytwarzania odmian uprawnych przeznaczonych do celów leczniczych. W nowoczesnej hodowli cykorii ważną cechą jest zdolność do klonalnego rozmnażania w kulturach *in vitro*. Celem naszych badań była ocena naturalnej populacji dzikiej cykorii pod względem zdolności do regeneracji roślin z eksplantatów liściowych. Oceniono wpływ 16 kombinacji różnych stężeń IAA i 2iP na indukcję kalusa i regenerację pędów z eksplantatów liściowych (0,5 cm^{-2}). Następnie, na pożywce uznanej za optymalną, porównano 25 roślin cykorii pod względem zdolności do tworzenia kalusa i regeneracji pędów. Potem badano wpływ różnych stężeń IAA na ukorzenianie pędów. Regeneracja pędów odbywała się głównie z udziałem kalusa, ale obserwowano też organogenezę bezpośrednią (8%). Największą efektywność regeneracji pędów stwierdzono na pożywce MS zawierającej 0,5 mg dm^{-3} IAA i 4 mg dm^{-3} 2iP – 97% eksplantatów wytworzyło pędy, ze średnią liczbą 15,5. Cechą wysoko odziedziczalną była ilość kalusa (h^2 = 0,83). Niższe współczynniki

odziedziczalności otrzymano dla liczby pędów na eksplantacie (0,55) i masy średniego pędu (0,40). Pędy dzikiej cykorii łatwo się ukorzeniały. Liczba korzeni i ich masa rosły wraz ze wzrostem stężeń IAA.

Słowa kluczowe: *Cichorium intybus*, rozmnażanie *in vitro*, organogeneza, współczynniki odziedziczalności

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