EXPERIMENTAL PAPER

Application of multi-shoots cultures in micropropagation of willow herb (*Chamaenerion angustifolium* (L.) Scop.)

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Summary

Introduction: Willow herb (*Chamaenerion angustifolium* (L.) Scop. syn. *Epilobium angustifolium* L. from *Onagraceae* family) is a valuable medicinal plant that has been used in the treatment of urogenital disorders including BPH (Benign Prostatic Hypertrophy). The raw material is a rich source of polyphenols as well as steroids, triterpenoids and fatty acids. Due to frequent interspecific hybridization, plants collected in wild display a very diverse and variable content of active compounds. This poses a challenge in obtaining high quality and homogenous raw material. Application of the *in vitro* cultures and micropropagation techniques may offer a solution for alternative methods of cultivation. **Objective:** This work presents preliminary results of the first implementation of *in vitro* cultures of willow herb to obtain material for medicinal purposes. **Methods:** Sterile seedlings were donors of explants, which were used for induction of multi-shoots culture according to a modified protocol described by Turker and co-workers. Statistical analysis was used for assessment of significance of differences among variables. **Results:** Six different genotypes (lines) originating from root explants were chosen for clonal propagation. Efficiency of the elaborated method was 16–20 shoots per explant. Finally, over 3000 acclimatized plants were obtained and used for field crops. **Conclusions:** The use of *Ch. angustifolium* *in vitro* cultures can contribute to the introduction of this valuable herb for field crops and increase the availability of the raw material for food and pharmaceutical industries.

Key words: willow herb, *in vitro* culture, micropropagation, multi-shoots cultures

INTRODUCTION

Willow herb (*Chamaenerion angustifolium* (L.) Scop. syn. *Epilobium angustifolium* L.) (*Onagraceae* family) can be found in Europe, Western Asia and North America. It grows in diverse habitats: edges of forests and clearings, along roadsides, in open mountainous areas as well as in the ruderal areas [1]. According to traditional folk medicine, its herb and roots were used as extracts and infusions in the treatment of gastrointestinal disorders, skin diseases and also of prostate, kidney and urinary tract disorders [2]. The raw material (*Epilobii herba*) is a rich source of polyphenols (flavonoids, phenolic acids and tannins), steroids, triterpenoids and fatty acids [3]. The most interesting group is macrocyclic ellagotannins represented by B and A oenothins and their derivatives. Oenothin B is a major constituent of plant material (2–14%) and has been regarded as one of main active compounds. The wide spectrum of pharmacological properties of willow herb has been documented: anti-androgen [4], anti-proliferative [5-7], anti-inflammatory and antioxidant [8], immunomodulatory [9, 10], antibacterial [11, 12] as well as analgesic properties [13]. Although activity of willow herb extracts has been contributed to the synergistic effect of tannins, flavonoids and other phenolic compounds, oenothin B has been found to be the main active compound. The inhibitory activity against 5- α -reductase and aromatase enzymes, which play a key role in the aetiology of BPH (Benign Prostatic Hypertrophy), have been reported [14]. Anti-proliferative activity of the *Epilobium* extracts has been revealed in the studies of Vitalone [5, 6]. The probable mechanism of the anti-proliferative activity as the effect of induction of neutral endopeptidase (NEP) has been established on various prostate cancer cells [15]. These studies showed that oenothin B specifically induced NEP in dose dependent manner and was responsible for the activity of the extract. Anti-inflammatory and antioxidant properties of oenothin B have been documented in subsequent research [8]. The studies on human prostate cancer cell lines (LNCaP) have confirmed anti-proliferative and anti-cancer effect of oenothin B and have proved the activity of its metabolites – urolithins [7, 16].

The studies on willow herb extracts have induced growing interest in therapeutic potential of the *Epilobium* plants and their application in the treatment or prevention of BPH and other diseases. On European market there are many products such as dietary supplements, medicinal products as well as cosmetic preparations with *C. angustifolium* extracts. A great interest in *Epilobium* plants has resulted in increasing demand on the raw material for the pharmaceutical and food industries. Nowadays, the majority of the raw material used for medicinal or food products is derived from crop plantations and only minority originate from the wild. It allows for obtaining large batches of high quality material and meeting the strict requirements for the medicinal products released on market. In case of *Ch. angustifolium*, the main problem is the need for obtaining raw material from the wild. Due to frequent interspecific hybridization, the wild plants display a diverse and variable content of active compounds. This poses a challenge in production of high quality and homogenous

raw material. Application of the *in vitro* cultures and micropropagation of selected genotypes offers an alternative way for traditional field cultivation. The results of this study are part of the project, in which *in vitro* cultures and micropropagation technique were used for the first time in order to obtain willow herb raw material for the production of a dietary supplement used in the BPH prevention.

The aim of this work is to present the preliminary results of implementing *in vitro* cultures in propagation of *Ch. angustifolium*.

MATERIAL AND METHODS

Chamaenerion angustifolium seeds obtained from the collection of the Garden of Medicinal Plants in Plewiska (Institute of Natural Fibres and Medicinal Plants) were used for induction of *in vitro* cultures. The seeds were sterilized by soaking in: 70% ethanol for 1 minute and then in ACE® solution (2:1) with a drop of Tween 20 (Sigma Aldrich) for 4 minutes, and washed three times in sterile water. The seeds were placed on Petri dishes with MS medium (according to Murashige and Skoog [17], with 30 g/l sucrose and 8 g/l Difco Bactoagar) without plant growth regulators. The percentage of germinated seeds was 99% within 7 days. After 6 weeks of incubation 15 seedlings were cut for explants: roots (1.0 cm), leaves (0.5 cm²), stem segments (0.25 cm) and shoot tips (0.25 cm). The explants were placed on induction medium according to the method followed by Turker's team [18] with slight modifications: MS micro-, macronutrients and vitamins supplemented with: BAP (0.1 mg/l), IAA (0.5 mg/l), vitamin C (0.1 g/l), casein hydrolysate (0.5 g/l), sucrose 30 g/l and agar 8/l (pH=5.7). Each seedling was marked by a number and derived multi-shoots and shoots from individual seedlings were represented by separated lines (genotypes). After four weeks of incubation, obtained multiple-shoots were subcultured on fresh induction medium and at the same time the shoots were individually separated and transferred into Magenta vessels (Sigma Aldrich) containing rooting medium: ½ MS with IAA (0.25–1.0 mg/l) with vitamin C (0.1 g/l). Secondary explants (multi-shoots) were subcultured every four weeks, and shoots were separated and transferred into the rooting medium. Multi-shoots consisted of approximately 16 lateral meristems per explant. For the assessment of regenerative potential the following experiment was conducted. A number of shoots per explant was calculated from each ten multi-shoots by counting individually separated shoots in three subsequent passages (21, 22 and 23). The shoots were qualified as 1 cm length and with minimum three developed leaves. The percentage of rooted plants was recorded after four weeks for 100 plants and the counting was repeated three times. All cultures were incubated in temperature 25°C under the 16/8 h photoperiod (cool – white fluorescent lights 25–30 μmol m⁻²s⁻¹).

The rooted plants were transferred into soil and perlite substrate (Kekkila Papperpot) and acclimatized in closed tunnels in the greenhouse conditions (21°C) for two weeks. The plants were hardening in the open tunnels in temperature 16°C for two weeks and for another two weeks in field conditions.

Statistical analysis

Statistical analysis for comparison of average number of shoots per explant were performed using Tukey test with the level of significance set at $p \leq 0.05$. Pearson's chi-square test was used for assessment of statistically significant difference of percentage of rooted shoots. All calculations were performed using Statistica 12 software.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS

The best shoot regeneration was obtained from stem fragments (96%) and root explants (60%), which formed multi-shoots (tab. 1). The rest of explants (leaves and shoot tips) have regenerated shoots only occasionally, more likely forming roots and callus. Percentage of survived explants varied and depended on explant type. Maximum survival rate was recorded for roots (100%) and the lowest percentage was noted for shoot tips (80%). Browning of tissues, especially stem segments and leaves leading to necrosis of explants was observed on induction medium in the subsequent passages. Due to this unfavorable effect, only multi-shoots obtained from root explants were chosen for further clonal propagation. Finally, six different genotypes (lines) originated from individual seedlings were subcultured for over 6 months (passages numbers from 2 to 23). The numbers of shoots and multi-shoots (calculated for all lines) changed between single subcultures (passages) (fig. 1). The increasing numbers of shoots and multi-shoots was noted up to 12th passage when over 1000 shoots were recorded. The number of secondary explants (multi-shoots) was increasing from 32 explants (2nd passage) up to 89 (12th passage) and then the number oscillated between 55 and 67 explants. The efficiency of shoot production was variable from passage to passage (fig. 2). The number of shoots per explant was increasing and reached maximum value (19.8 ± 4.503) between 11th and 13th passage, then the obtained shoots oscillated between 14 and 17 (fig. 2).

Table 1.

Induction of shoots from different types of explants

Explants	Number of explants	Explants forming shoots [%]	Survived explants [%]
Roots	55	60	100
Stem fragments	24	100	96
Leaves	123	10.7	83.7
Shoot tips	15	25	80

Figure 1.
The number of shoots and multi-shoots obtained in every second passage (No. 2 to 22)

Table 2.
Effects of different IAA concentrations on root formation from regenerated shoots

IAA Concentration [mg/l]	Rooted plants [%]
0.25	96.6±2.10
0.5	98.0±1.01
1.0	99.0±1.01

Results recorded after four weeks and expressed as % of vigorous rooted plants ± SD. No statistically significant difference was found.

Figure 2.
Average number of shoots per secondary explant (multi-shoot). Error bars express SD

In a separate experiment, average numbers of shoots per explant were calculated during subsequent three passages to determine the regenerative potential of the individual genotypes (lines). The best result was observed for PL-58 line – 20.4 ± 3.872 shoots per explant (fig. 3). Average numbers of shoots calculated for explant varied between genotypes and oscillated between 16.1 and 20.4 (fig. 3). Statistically significant differences were noted for lines PL-37, PL-58 and PL-38 (fig. 3).

Figure 3.

Average number of shoots per secondary explant (multi-shoot) of individual lines. Error bars express SD. Data followed by different letters are significantly different at $p \leq 0.05$ according to Tukey test (one-way Anova)

The shoots were rooted on medium containing three different concentrations of IAA (0.25 mg/l; 0.5 mg/l and 1.0 mg/l). Although no statistically significant differences between IAA concentration were noted, higher concentration of auxin (1.0 mg/l) caused callusing, which had unfavorable effect on the appearance of the plantlets. The optimal concentration of IAA was 0.5 mg/l and 98% of vigorous and rooted plants were recorded.

The whole cycle from seedlings to acclimatized plants was 10 to 12 weeks. Acclimatization resulted in high survival rate (98%) of rooted and hardened plantlets. Finally, over 3,000 acclimatized plants were obtained and used for field crops.

DISCUSSION

This work presents the results of preliminary studies on the implementation of multi-shoots cultures in willow herb propagation for raw material production. According to the best of our knowledge there are no reports dedicated to the application of *Ch. angustifolium* *in vitro* cultures in propagation and cultivation.

Literature concerning micropropagation of *Ch. angustifolium* is extremely scarce and includes only one report published by Turker and co-workers in 2008 [18]. There are only a few papers on *in vitro* propagation of related species: *E. parviflorum* and *E. hirsutum* [19-21]. Turker's protocol describes rapid and efficient induction of shoots from seedling explants *via* direct organogenesis. They obtained the best shoot proliferation for root explants on MSMO medium (Murashige and Skoog's minimal organics) [17] with BAP (0.1 mg/l) and IAA (0.5 mg/l) supplemented with vitamin C. Maximum recorded number of shoots per explant was 37.25 ± 1.9 and the whole cycle (from explant to acclimatized plants) lasted 10 weeks.

In the presented study, micropropagation protocol elaborated by Turker's team was used with some modifications. The main difference was continuous propagation using secondary explants (multi-shoots), while Turker obtained shoots from primary explants (roots). Another difference was related with the scale of the experiments. Turker and co-workers performed experiments on a small number of explants in the laboratory scale. The presented results concern production and acclimatization of more than 3000 plantlets, what implies mass propagation. Minor differences related to media composition: induction medium was based on MS (according to Murashige and Skoog 1962) instead of MSMO and supplemented with both vitamin C (0.1 mg/l) and casein hydrolysate (0.5 g/l), which were absent in the original protocol. Rooting medium contained only half the dose of micro- and macronutrients and vitamins of MS medium with the same concentration of auxin (0.5 mg/l IAA).

Variable regeneration potential of the explants was observed at the induction stage (tab. 1). The best results were recorded for stem fragments and roots, although only the root explants formed multi-shoots, contrary to the explants originated from stems, which were browning and died in the subsequent passages. In general, our observations are consistent with Turker's findings that roots are the first choice explants due to the greatest regenerative potential, but some differences have been noted. In Turker's experiment stem explants did not produce shoots at all. Leaves however, were second in terms of shoot formation contrary to our findings where they produced shoots only occasionally and more often died. In our study we used 15 objects (seedlings) and we observed genotype dependent response of explants expressed in callusing and especially in browning tissue.

The browning of tissues was also noted by Turker's team and was considered as the major problem, which limited the number and quality of the regenerated shoots. The browning of tissues is an effect of polyphenol oxidation released from cut explants and formation of quinones, which are highly reactive and toxic for plant tissues [22, 23]. This unfavourable physiological reaction is often a challenge in establishment of *in vitro* cultures, especially in case of phenolic compound abundant species. To minimize browning of explants some antioxidants [23, 24] or absorbents, like activated charcoal or polyvinylpyrrolidone (PVP), are added into media [25]. Some authors suggest pre-soaking of explants in antioxidant mixture to

prevent oxidation of polyphenols [26, 21]. By adding vitamin C (100 mg/l) into all prepared media Turker and his co-workers eliminated the problem. Vitamin C is commonly known as an antioxidant but it is light- and thermo-labile. Antioxidant effect of vitamin C was not satisfactory in our study. Supplementation of the induction medium with both vitamin C and casein hydrolysate limited the problem but did not eliminate it completely. It is known that casein hydrolysate contains peptides with antioxidant activity [27, 28], which could help reduce browning of tissues.

Efficiency of micropropagation is usually determined by numbers of shoots produced by a single explant. A high number of shoots (37.25 ± 1.9) was recorded by Turker for root explants and this is the best result for this species and other plants from *Onagraceae* family. The average number of shoots recorded per explant was lower (maximum 20.4 ± 3.87) in this study. The efficiency depended on the passage and the genotype (fig. 2, 3). The regenerative potential could result from different ability of plant material and also was determined by differences in the applied micropropagation technique. In Turker's experiment the number of shoots was calculated after three weeks of incubation and then proliferating explants were transferred into elongation medium (MS without growth regulators). In our study we omitted the elongation stage and subcultured multi-shoots on induction medium, counting the number of regenerated shoots and secondary explants for a single passage. It is difficult to compare the results due to different processing during micropropagation. It is worth mentioning that shoots and multi-shoots were obtained simultaneously, what had an effect on lower multiplication rate but it allowed for continuing propagation of selected genotypes. The regenerative potential of lines regarding the number of shoots recorded per explant oscillated between 16.1 and 20.4 (fig. 3). Some genotypes differed significantly (PL-37/PL-58 and PL-38/PL-58) in shoots regeneration. Differences in shoot production may also be affected by multi-shoots adaptation to continuous subculturing and reduced exudation of polyphenols into medium.

Acclimatization is often a critical stage of *in vitro* propagation. Thanks to application of closed tunnels and treatments of the soil with microorganism mixture for improving the physiological state of plantlets, a high percentage (98.7%) of acclimatized plants was achieved. This enabled to establish a field culture and obtain the raw material. The implemented clonal propagation of willow herb is an efficient and rapid method, which resulted in production of over 3,000 plants. This is a very good predictor and allows for concluding that the use of *in vitro* culture can contribute to the introduction of willow herb for field crops and thus increase the availability of the raw material for food and pharmaceutical industries.

In spite of satisfactory results of the preliminary studies, we should be aware of some limitations of this method: dependence on a genotype and adaptability to the *in vitro* conditions. We can clone only those individuals which are responsive to BAP and IAA. Moreover, continuous propagation *via* multi-shoot cultures is associated with the risk of somaclonal variation and can lead to the natural aging of

cultures resulting in the loss of regenerative ability. Therefore, we should search for new methods of micropropagation and develop techniques for germplasm repository for preservation of valuable genotypes.

CONCLUSIONS

The first attempt of *Ch. angustifolium* micropropagation on massive scale resulted in production of over 3,000 plantlets. The modified Turker's protocol is an efficient and rapid method, which allows for obtaining of a high number of shoots per explant (16–20). Genotype-dependent response of the explants was observed during regeneration. The major problem was browning of tissues and necrosis of the explants, what limited the number and quality of the regenerated shoots. The use of *Ch. angustifolium* *in vitro* cultures can contribute to the introduction of this valuable herb species for field crops and increase the availability of the raw material for food and the pharmaceutical industries.

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REFERENCES

1. McColl J. Willowherb (*Epilobium angustifolium* L.): biology, chemistry, bioactivity and uses. *Agro Food Ind Hi Tec* 2002; 50:239-247.
2. Vogl S, Picker P, Mihaly-Bison J, Fakhrudin N, Atanasov AG, Heiss EH et al. Ethnopharmacological *in vitro* studies on Austria's folk medicine – unexplored *in vitro* anti-inflammatory activities of 71 Austrian traditional herbal drugs. *J Ethnopharmacol* 2013; 149:750-771. doi: <http://dx.doi.org/doi:10.1016/j.jep.2013.06.007>
3. Granica S, Piwowarski JP, Czerwińska ME, Kiss AK. Phytochemistry, pharmacology and traditional uses of different *Epilobium* species (*Onagraceae*): A review. *J Ethnopharmacol* 2014; 156:316-346. doi: <http://dx.doi.org/doi:10.1016/j.jep.2014.08.036>
4. Hiermann A, Burcar F. Studies of *Epilobium angustifolium* extracts on growth of accessory sexual organs in rats. *J Ethnopharmacol* 1997; 55:179-183. doi: [http://dx.doi.org/doi:10.1016/S0378-8741\(96\)01498-5](http://dx.doi.org/doi:10.1016/S0378-8741(96)01498-5).
5. Vitalone A, Bordini F, Baldazzi C, Mazzanti G, Tita B. Antiproliferative effect on a prostatic epithelial cell line (PZ-HPV-7) by *Epilobium angustifolium* L. *IL Farmaco* 2001; 56:483-489. doi: [http://dx.doi.org/doi:10.1016/S0014-827X\(01\)01067-9](http://dx.doi.org/doi:10.1016/S0014-827X(01)01067-9)

6. Vitalone A, McCroll J, Thome D, Costa LG, Tita B. Characterization of the effect of *Epilobium* extract on human cell proliferation. *Pharmacology* 2003; 69:79-87. doi: <http://dx.doi.org/doi:10.1159/000072360>
7. Stolarczyk M, Naruszewicz M, Kiss AK. Extracts from *Epilobium* sp. herbs induce apoptosis in human hormone-dependent prostate cancer cells by activating the mitochondrial pathway. *J Pharm Pharmacol* 2013; 65:1044-1054. doi: <http://dx.doi.org/doi:10.1111/jphp.12063>
8. Kiss AK, Bazylko A, Filipek A, Granica S, Jaszewska E, Kiarszys U et al. Oenothien B's contribution to the anti-inflammatory and antioxidant activity of *Epilobium* sp. *Phytomedicine* 2011; 18:55-560. doi: <http://dx.doi.org/doi:10.1016/j.phymed.2010.10.016>
9. Ramstead AG, Schepetkin I, Quinn MT, Jutila MA. Oenothien B, a cyclic dimeric ellagitannin isolated from *Epilobium angustifolium*, enhances IFN γ production by lymphocytes. *Plos One* 2012; 7(11)e50546. doi: <http://dx.doi.org/doi:10.1371/journal.pone.0050546>
10. Ramstead AG, Schepetkin I, Todd K, Loeffelholz J, Berardinelli JG, Quinn MT et al. Aging influences the response of T cells to stimulation by the ellagitannin, oenothien B. *Int Immunopharmacol* 2015; 26(2):367-377. doi: <http://dx.doi.org/doi:10.1016/j.intimp.2015.04.008>
11. Rauha JP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala L et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *Int J Food Microbiol* 2000; 56:3-12. doi: [http://dx.doi.org/doi:10.1016/S0168-1605\(00\)00218-X](http://dx.doi.org/doi:10.1016/S0168-1605(00)00218-X)
12. Bartfay WJ, Bartfay E, Jhonson JG. Gram-negative and Gram-positive antibacterial properties of the whole plant extract of willow herb (*Epilobium angustifolium*). *Biol Res Nurs* 2012; 14:85-89. doi: <http://dx.doi.org/doi:10.1177/1099800410393947>
13. Tita B, Abdel-Haq H, Vitalone A, Mazzanti G, Saso I. Analgesic properties of *Epilobium angustifolium*, evaluated by the hot plate test and the writhing test. *IL Farmaco* 2001; 56:31-343. doi: [http://dx.doi.org/doi:10.1016/S0014-827X\(01\)01046-1](http://dx.doi.org/doi:10.1016/S0014-827X(01)01046-1)
14. Durcey B, Marston A, Gohring S, Hartmann RW, Hostettmann K. Inhibition of 5 α -reductase and aromatase by the ellagitannins oenothien A and oenothien B from *Epilobium* species. *Planta Med* 1997; 63:111-114.
15. Kiss A, Kowalski J, Melzig MF. Induction of neutral endopeptidase activity in PC-3 cells by an aqueous extract of *Epilobium angustifolium* L. and oenothien B. *Phytomedicine* 2006; 13:284-289. doi: <http://dx.doi.org/doi:10.1016/j.phymed.2004.08.002>
16. Stolarczyk M, Piwowarski JP, Granica S, Stefańska J, Naruszewicz M, Kiss AK. Extracts from *Epilobium* sp. herbs, their components and gut microbiota metabolites of *Epilobium* ellagitannins, urolithins, inhibit hormone-dependent prostate cancer cells (LNCaP) proliferation and PSA secretion. *Phytother Res* 2013; 27: 1842-1848. doi: <http://dx.doi.org/doi:10.1002/ptr.4941>
17. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures *Physiol Plantarum* 1962; 15(3):473-497. doi: <http://dx.doi.org/doi:10.1111/j.1399-3054.1962.tb08052.x>
18. Turker AU, Mutlu EC, Yildirm AB. Efficient *in vitro* regeneration of fireweed, a medicinal plant. *Acta Physiol Plant* 2008; 30(4):421-426.
19. Vălimăreanu S, Deliu C. Polymorphism detection of *in vitro* cultivated *Epilobium* (*Onagraceae*) species using RAPD method. *Contributii Botanice Cluj-Napoca* 2008; 43:113-120.
20. Tămas M, Toiu A, Oniga I, Deliu C, Oltean B, Coldea GH. Quantitative determination of polyphenols and flavonoids from indigenous species of *Epilobium* of wild origin and *in vitro* regenerated plantlets. *Contributii Botanice Cluj-Napoca* 2009; 44:119-123.
21. Deliu C, Coste A, Mircea T. *Epilobium* Sp. (Willow Herb): Micropropagation and Production of Secondary Metabolites, [in:] *Biotechnology for Medicinal Plants*, (ed.) S. Chandra Springer-Verlag Berlin Heidelberg 2013. doi: http://dx.doi.org/doi:10.1007/978-3-642-29974-2_6
22. Gacche RN, Shete AM, Dhole NA, Ghole VS. Reversible inhibition of polyphenol oxidase from apple using L-cysteine. *Indian J Chem Technol* 2006; 13:459-463.
23. Misra P, Toppo, DD, Gupta N, Chakrabarty D, Tuli R. Effect of antioxidants and associate changes in antioxidant enzymes in controlling browning and necrosis of proliferating shoots of elite *Jatropha curcas* L. *Biomass Bioenerg* 2010; 34: 1861-1869. doi: <http://dx.doi.org/doi:10.1016/j.biombioe.2010.07.027>
24. Ngomuo M, Mneney E, Ndakidemi P. Control of lethal browning by using ascorbic acid on shoot tip cultures of a local *Musa* ssp. (Banana) cv. Mzuzu in Tanzania. *Afr J Biotechnol* 2014; 13(6):1721-1725. doi: <http://dx.doi.org/doi:10.5897/AJB2013.13251>

25. Li Y, Wang X, Chen J, Cai N, Zeng H, Qiao Z et al. A method for micropropagation of *Cornus wilsoniana*: An important biofuel plant. *Ind Crop Prod* 2015; 76:49-54. doi: <http://dx.doi.org/doi:10.1016/j.indcrop.2015.06.042>
26. Zentkeler E, Kwaśna H. Pre-treatment of *Dryopteris cristata* (L.) A Gray rhizome as a method of elimination of contaminants and explant browning micropropagation. *Biodiv Res Conserv* 2007; 5(8):81-86.
27. Suetsuna K, Ukeda H, Ochi H. Isolation and characterization of free radical scavenging activities peptides derived from casein. *J Nutr Biochem* 2000; 11(3):128-131. doi: [http://dx.doi.org/10.1016/S0955-2863\(99\)00083-2](http://dx.doi.org/10.1016/S0955-2863(99)00083-2)
28. Irshad I, Kanekanian A, Peters A, Masud T. Antioxidant activity of bioactive peptides derived from bovine casein hydrolysate fractions. *J Food Technol* 2015; 52(1):231-239. doi: <http://dx.doi.org/doi:10.1007/s13197-012-0920-8>

ZASTOSOWANIE KULTUR WIELOROŚLINEK W NAMNAŻANIU WIERZBÓWKI KIPRZYCY (*CHAMAENERION ANGUSTIFOLIUM* (L.) SCOP.)

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Streszczenie

Wstęp: Ziele wierzbówki koprzy *Chamaenerion angustifolium* (L.) Scop. syn. *Epilobium angustifolium* L. (rodzina *Onagraceae*) stanowi cenny surowiec zielarski w leczeniu schorzeń układu moczowo-płciowego, w tym także łagodnego przerostu prostaty (ang. *Benign Prostatic Hypertrophy*, BPH). Surowiec zawiera liczne związki fenolowe, jak również steroidy, triterpenoidy i kwasy tłuszczowe. Niezwykle łatwe krzyżowanie się wierzbówki z innymi gatunkami pokrewnymi prowadzi do powstania mieszańców o bardzo zróżnicowanej i zmiennej zawartości związków aktywnych, co nastęrcza trudności w uzyskaniu wyrównanego surowca o wysokiej jakości. Alternatywnym rozwiązaniem może być zastosowanie mikropropagacji do otrzymywania i namnażania wyselekcjonowanych genotypów w kultu-

rach *in vitro*. **Cel:** Praca przedstawia wstępne wyniki badań, gdzie po raz pierwszy zastosowano kultury *in vitro* do namnażania wierzbówki kiprzycy w celu pozyskania surowca do produkcji suplementu diety. **Metody:** Do inicjacji kultur wieloroślinek (multi-shoots) wg zmodyfikowanej metody Turkera (2008) posłużyły eksplantaty pochodzące ze sterylnych siewek. Analizy statystycznej użyto do określenia istotności różnic zmiennych. **Wyniki:** Do klonalnego namnażania wybrano sześć różnych linii (genotypów) pochodzących z eksplantatów korzeniowych. Wydajność opracowanej metody wynosiła 16–20 pędów na eksplantat. Ostatecznie otrzymano ponad 3000 zaaklimatyzowanych roślin, które posłużyły do założenia upraw polowych. **Wnioski:** Zastosowanie kultur *in vitro* wierzbówki kiprzycy może przyczynić się do zwiększenia dostępności tego surowca zielarskiego dla celów zarówno przemysłu spożywczego, jak i farmaceutycznego.

Słowa kluczowe: wierzbówka kiprzyca, kultury *in vitro*, mikropropagacja, kultury wieloroślinek