

ELABORATION OF MICROPROPAGATION SYSTEM OF MEDICINAL PLANT YACON (*Smallanthus sonchifolius* (Poepp.) H. Rob.)

Waldemar Kiszczak¹✉, Urszula Kowalska¹, Maria Burian², Sława Glińska³, Marcin Domaciuk⁴, Krystyna Górecka¹

¹ Department of Applied Biology, The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

² Department of Plant Anatomy and Cytology, Faculty of Biology, University of Warsaw, Miecznikowa 1, 02-096 Warsaw, Poland

³ Laboratory of Microscopic Imaging and Specialized Biological Techniques Faculty of Biology and Environmental Protection, University of Lodz, Banacha 12/16, 90-237 Lodz, Poland

⁴ Institute of Biological Sciences, Department of Cell Biology, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland

ABSTRACT

Yacon becomes an important plant with strong medical and dietary potential. Therefore, studies on the optimization of yacon micropropagation were undertaken. Reproduction of yacon is conducted by traditional methods with green seedlings or long offsets taken from the underground and aboveground rootstock. The influence of different disinfection methods, various initial explants and growth regulators, their concentrations, and combinations on the efficiency of micropropagation have been analyzed. The most effective disinfection was the use of 70% ethanol and 0.5% Tween 20. The optimal initial explants were apical and lateral buds cultured on Murashige and Skoog (MS) medium with 0.2 mg L⁻¹ kinetin and 1 mg L⁻¹ indole-3-acetic acid. The most intensive shoot regeneration occurred on MS medium with 1 mg L⁻¹ thidiazuron and 1-naphthylacetic acid. Histological observations demonstrated the strong effect of growth substances on shoot organogenesis from callus tissue. Shoots obtained from *in vitro* cultures rooted easily on modified MS media with ½ or ¼ concentration of macroelements, 20 g L⁻¹ sucrose and 3 mg L⁻¹ 1-naphthylacetic acid. All plants adapted to *ex vitro* conditions.

Key words: auxins, cytokinins, histological structure, micropropagation, regeneration, tissue culture

Abbreviations: FOS – fructooligosaccharides; BA – 6-benzylaminopurine; Kin. – kinetin; TDZ – thidiazuron; 2iP – N6-(2-isopentenyl)adenine; IAA – indole-3-acetic acid; IBA – indole-3-butyric acid; NAA – 1-naphthylacetic acid; PGRs – plant growth regulators

INTRODUCTION

Due to their contribution to the reduction of disease risk, health improvement and well-being foodstuffs with beneficial influence on the human health are acquiring growing importance. Yacon (*Smallanthus sonchifolius* (Poepp. and Endl.) H. Robinson) is among the plants belonging to the group of functional health food. Edible tubers of yacon contain many valuable

minerals, vitamins, and prebiotic dietary fibers, which stimulate selective growth and beneficial intestinal microflora activity [Lachman et al. 2003, Passos and Park 2003, Lago et al. 2011]. Prebiotic compounds exert positive influence on immune system, enhancing the resistance to infections and allergic reactions and lowering the risk of cancer and diabetes. In addition to

the prebiotic compounds, yacon contains flavonoids, phenolic acids and the amino acid tryptophan, which possess antioxidant, anti-inflammatory, antibacterial, and anticarcinogenic properties. Phenolic compounds in yacon protect biomolecules such as DNA, lipids or proteins from damage caused by free oxygen and nitrogen radicals [Corrêa et al. 2009].

The plants comprising compounds with beneficial influence on human health are significant also for plant breeding and food trade. As a source of health promoting ingredients yacon is gaining an increasing consumer demand [Millela et al. 2011]. Extracts from yacon roots are mainly incorporated into products like flour, juices, purees, chips, sweeteners in the form of syrups with a high content of fructooligosaccharides. Moreover, yacon has a huge potential to become a valuable crop for small farming.

Low level of pollen germination and low seed viability make the sexual reproductivity of yacon very difficult [Mansilla et al. 2010]. Therefore, yacon is mostly reproduced vegetatively by cuttings or tuber division. However, these reproduction methods may cause bacterial and viral pathogen transfer [Corrêa et al. 2009]. The threat of disease transmission at the vegetative propagation poses a necessity to elaborate a method of *in vitro* multiplication. The development of protocol for *in vitro* propagation of yacon is expected to provide a possibility for more extensive research on acquiring active substances from yacon cultures and their application in pharmaceutical, cosmetic or food industry for producing diet supplements.

The studies on the micropropagation of Yacon in *in vitro* cultures were conducted by: Matsubara et al. [1990], Hamada et al. [1990], Estrella and Lazarte [1994], Matsubara [1997], Niwa et al. [2002] and Mogor et al. [2003]. In the above-mentioned publications authors tested various types of sterilization methods of initial explants. Different parts of Yacon and various media were used to establish *in vitro* cultures. However, none of the cited publications describe the whole process of micropropagation and the elaboration of technology of rapid multiplication in *in vitro* cultures.

The aim of this study was to develop an efficient method of *in vitro* propagation of yacon, including all stages of the process. Methods for disinfection, use of various initial explants and the influence of selected

plant growth regulators (PGRs) on induction of regeneration, proliferation and rooting are analyzed. Histological analyzes of tissue formation are also presented.

MATERIALS AND METHODS

Study area

Studies were conducted in The National Institute of Horticultural Research, Poland. The influence of individual factors at each stage: induction, regeneration, multiplication and acclimatization were analyzed.

Research protocol

Experiment I – disinfection methods. The following disinfection methods were applied: I. 70% (v/v) ethanol + 0.5% (v/v) Tween 20 for 2 min., rinsed 10 times with sterile water; II. 70% ethanol + 0.5% Tween 20 for 2 min., 10% calcium hypochlorite + 0.5% Tween 20 for 30 min., rinsed 10 times with sterile water; III. 0.1% HgCl₂ + 0.5% Tween 20 for 10 min., rinsed 10 times with sterile water, 10% chloramine solution + 0.5% Tween 20 for 20 min., rinsed 10 times with sterile water.

In the first experiment, leaves collected from plants grown in a greenhouse were used as initial explants in the first experiment, where 3 methods of sterilization were tested. First 2 methods of disinfection were applied in the second experiment, where meristems and leaves were used as initial explants. In the current experiment the effect of sterilization method on the purity of *in vitro* cultures and the type of obtained multiplication was examined.

Experiment II – regeneration. The experiments were conducted on MS medium [Murashige and Skoog 1962] containing 20 g L⁻¹ sucrose, 6 g L⁻¹ agar with pH 5.6. The following cytokinins were applied: BA, Kin., TDZ, 2iP. The auxins were: IAA, IBA, NAA. Various combinations of cytokinins, auxins and putrescine were tested (Tab. 1).

The explants such as: A) leaves and leaf fragments; B) nodal and internodal shoot fragments; C) shoot tips; D) internodal fragments of axillary buds; E) nodal fragments of axillary buds; F) flower buds were placed in tubes, in a phytotron at 20°C with a 16/8 photoperiod under fluorescent light at 30 μmol m⁻² s⁻¹. The observations were done twice a week for 2 months. Protocorm-Like Bodies (PLBs)

obtained in the first passage were then transferred on the same set of media.

Experiment III – rooting and acclimatization. Shoots obtained on the regeneration media (Tab. 1) were transferred onto the MS rooting media, which contained ½ or ¼ concentration of macroelements with the standard amount of microelements for this medium (marked as MS ½ or MS ¼) with sucrose (20 g L⁻¹) and NAA (1 mg L⁻¹).

Complete plants were planted in multipots filled with sand and peat substrate (3 : 1). Plants were grown

in plastic tunnel, on capillary mat, in order to ensure about 100% humidity, in a growth chamber with controlled conditions: temperature of 18°C during the day and 16°C at night with a light intensity of approx. 30 μmol m⁻² s⁻¹ (16/8 hours light photoperiod) or in the greenhouse.

Cross sectional histological analyzes

Cross sections of yacon callus were prepared to analyse the histological structure. Semi-thin sections (1 μm) were cut with a glass knife on ultramicrotome

Table 1. Combinations of plant growth regulators (PGRs) and putrescine used in experiments on the micropropagation of yacon

Medium symbols	PGRs (mg L ⁻¹)							
	Kin.	IAA	BA	NAA	IBA	TDZ	2iP	putrescine
MS – A	0.2	1.0	–	–	–	–	–	–
MS – B	4.0	0.8	–	–	–	–	–	–
MS – C	1.0	1.0	–	–	–	–	–	–
MS – D	–	–	0.2	1.0	–	–	–	–
MS – E	0.2	1.0	5.0	–	1.0	–	–	–
MS – F	–	–	0.2	1.0	–	–	–	0.5
MS – G	–	–	0.2	1.0	–	–	–	1.0
MS – H	–	1.0	1.0	–	–	–	–	–
MS – I	–	–	2.0	–	1.0	–	–	–
MS – J	–	–	5.0	–	1.0	–	–	–
MS – K	–	–	1.0	0.1	–	0.1	–	–
MS – L	1.0	–	1.0	–	–	0.01	1.0	–
MS – M	–	–	–	1.0	–	1.0	–	–
MS – N	–	–	–	1.0	–	2.0	–	–
MS – O	–	–	–	1.0	–	5.0	–	–
MS – P	–	–	–	1.0	–	10.0	–	–
MS – Q	–	–	–	1.0	–	20.0	–	–
MS – R	–	–	–	1.0	–	–	2.5	–
MS – S	–	0.01	–	–	–	–	22.0	–
MS – T	–	0.01	–	–	–	–	30.0	–
MS – U	–	0.01	–	–	–	–	40.0	–
MS – V	–	0.01	–	–	–	–	20.0	–
MS – W	–	0.01	0.2	1.0	–	–	30.0	–

(Ultracut, Reichert-Jung, Germany), stained with 1% toluidine blue and analyzed with light microscope (Eclipse 50i, Nikon, Japan) equipped with camera (Power Shot digital 640, Canon, Japan), and software for image analysis CoolView (PRECOPTIC).

Statistical analysis

Data were analyzed using non-parametric analyses such as the Kruskal-Wallis test (with Conover-Inman post hoc tests). Statistica v. 8.0. for Windows (Statsoft Inc. Tulsa, USA). The number of 10 explants, in 3 or 4 repetitions, were placed on each tested media in every explant category: leaves – 30 explants; leaf fragment – 40; flower buds – 30; nodal shoot fragments – 40; internodal shoot fragments – 40; shoot tips – 30; axillary buds – 30. The number of individual types of explants depended on their availability. Due to the various number of repetitions, a non-parametric test was applied.

RESULTS

Experiment I – disinfection methods

From all disinfection methods of initial explants, the treatment with method I, was the most efficient. And the method II appeared to be the least effective. The percentage of contaminated explants was very high. The third applied method (III) was too drastic for initial explants and caused up to 50% death (Tab. 2).

In the second experiment, buds or leaves were used as initial explants which were treated according to methods I and II. Leaf explants treated with method II of disinfection did not show any proliferation capacity.

In case of buds the disinfection effect of applied methods was similar to the above mentioned. Comparing the influence on the explant vitality, we established that the second method was less effective (Tabs 3 and 4).

Experiment II – regeneration

A – Initial explants – leaves, leaf fragments. The growth of specific tissues PLBs, in the total number of 515, was observed on leaves and their fragments cultured on media containing various combinations of growth regulators: MS – D; MS – F; MS – N; MS – M and MS – O (Fig. 1). The intensity of this process was higher on media supplemented with TDZ. Such tissues were still proliferating after transferring on the same media in the second passage, where the number of 855 PLBs was obtained. Adventitious shoot formation was observed after placing the tissues on MS – E (52 pieces) and MS – J medium (96 pieces). The most intensive regeneration was observed on the latter medium.

B – Initial explants – nodal and internodal shoot fragments. In the first passage, complete plants were obtained from nodal and internodal shoot fragments cultured on the following media: MS – B (118 pieces); MS – W (103 pieces); MS – F (92 pieces). The culture of internodal segments allowed to obtain well developed plants with proper root system. The intensive production of new shoots was observed for the same initial explants cultured on the MS – J medium (364). The formation of the specific tissue (PLBs), similar to the tissues growing on leaves, was obtained (66) after placing internodal segments on MS – M medium.

C – Initial explants – shoot tips. Complete 35 plants were obtained from shoot tips on MS-A medium and 51 were obtained on MS-J medium. Plants were planted directly into substrate for adaptation to *ex vivo* conditions. On MS-A medium, 3 unrooted shoots were obtained, whereas the number of 99 was received on MS-J medium. All shoots were transferred onto the rooting media. Also, 3 shoot buds were obtained on MS-A medium. The most regeneration (5.9) per 1 initial explant, among 2 categories of initial explants,

Table 2. The influence of disinfection methods on the purity of explants and their survival

Disinfection methods	Explants							
	plated		contaminated		clean		dead	
	no		no	%	no	%	no	%
I	23		7	30.4	15	65.2	1	4.4
II	26		16	61.5	7	26.9	3	11.6
III	28		1	3.6	13	46.4	14	50.0

Table 3. The influence of disinfection on the purity of explants

Initial explant	Disinfection methods					
	I			II		
	used	clean		used	clean	
	no	no	%	no	no	%
Meristems	61	52	85.3	60	50	83.3
Leaves	20	19	95	20	20	100

Table 4. The influence of disinfection method on the frequency of initial explants responses

Initial explant	Regeneration type	Disinfection methods / frequency of initial explants responses (%)	
		I	II
Meristems	plants	1.9	0
Leaves		0	0
Meristems	single unrooted shoots	1.9	1
Leaves		0	0
Meristems	shoot multiplication	11.5	0
Leaves		0	0
Meristems	adventitious buds	40.5	10
Leaves		0	0
Meristems	large callus tissue*	38.5	20
Leaves		12	0
Meristems	little callus tissue**	5.7	14
Leaves		0	0
Meristems	no response	0	5
Leaves		7	20

* Large callus tissue – callus weighing more than 1 g

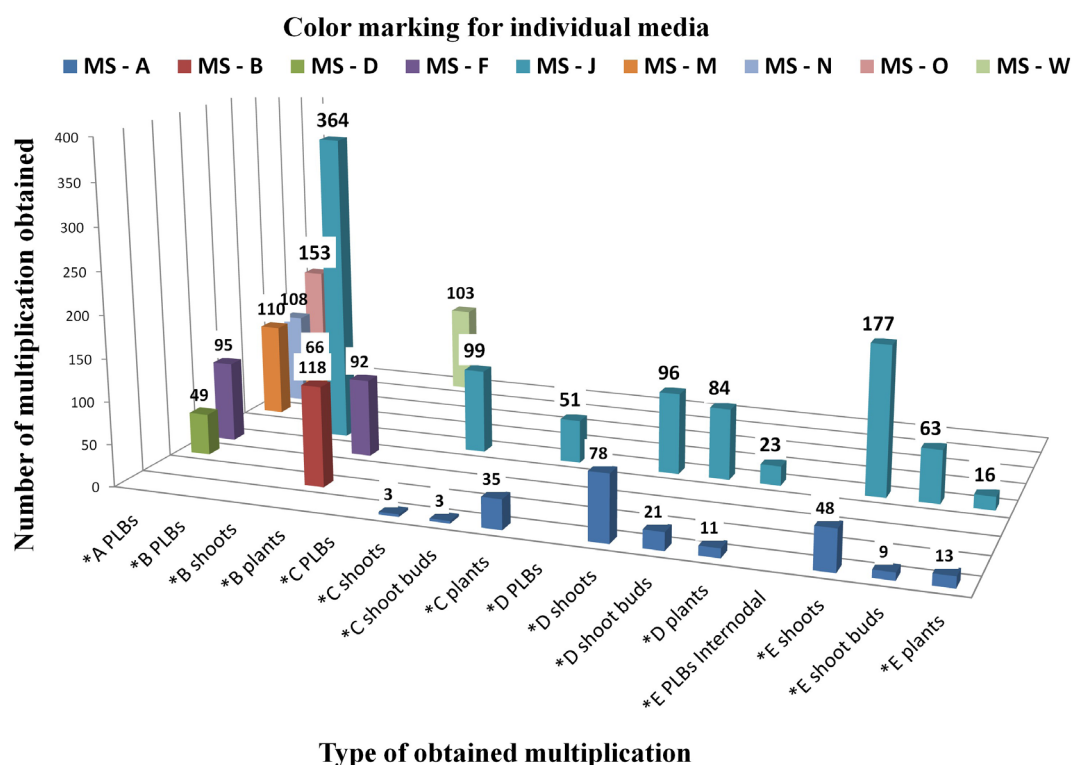
** Little callus tissue – callus weighing less than 1 g

was obtained from internodal fragments of axillary buds on MS-J medium (Tab. 5). No regeneration was achieved on the remaining media from both analyzed types of initial explants.

D – Initial explants – nodal fragments of axillary buds. When the nodal fragments of axillary buds were used as initial explants, plants were obtained on MS-A medium in the amount of 11 pcs. and 23 pcs. on MS-J medium. On the same media, shoots were obtained in the number of 78 pcs. on MS-A medium and 96 pcs. on the second medium. Also, the multiplication classified as shoot buds in the number of 21 pcs. was obtained on MS-A medium and 84 pcs. on MS-J medium.

E – Initial explants – intermodal fragments of axillary buds. In case of the nodal fragments of axillary buds, similarly to other initial explants such as shoot tips and nodal fragments of axillary buds, the same categories of multiplication were obtained on MS-A medium. The following number was received: plants – 13 pcs.; shoots – 48 pcs. and shoot buds – 9 pcs. On the MS-J medium, the following amount was obtained: plants – 16 pcs; shoots – 177 pcs. and shoot buds 63 pcs.

Due to the fact that the same types of multiplication were obtained on the same media for the 3 above-mentioned categories, a statistical comparison of the



- * A – initial explants: leaves, leaf fragments
- * B – initial explants: nodal and internodal shoot fragments
- * C – initial explants: shoot tips
- * D – initial explants: axillary buds: nodal fragments
- * E – axillary buds: internodal fragments

Fig. 1. Number and the type of obtained multiplication from various types of initial explants on individual media. The graph does not present initial explants and media on which multiplication was not obtained

Table 5. The number of shoots and shoot buds obtained on MS media from different explants depending on the plant growth regulators (PGRs) combination

Initial explants	Medium: PGRs (mg L ⁻¹)	Per 1 initial explant	
		shoots	shoot buds
Shoot tips	MS – A (Kin 0.2 + IAA 1.0)	1.1 a	0.1 a
	MS – J (BA 5.0 + IBA 1.0)	3.3 b	5.5 d
Auxiliary buds	MS – A (Kin 0.2 + IAA 1.0)	2.6 ab	0.7 abc
	MS – J (BA 5.0 + IBA 1.0)	3.2 bc	2.8 cd
internodal fragments	MS – A (Kin 0.2 + IAA 1.0)	1.6 ab	0.3 ab
	MS – J (BA 5.0 + IBA 1.0)	5.9 c	2.1 bcd

Means for each explants type marked with the same letter do not differ significantly from each other at a significance level of $\alpha = 0.05$; Kruskal-Wallis test

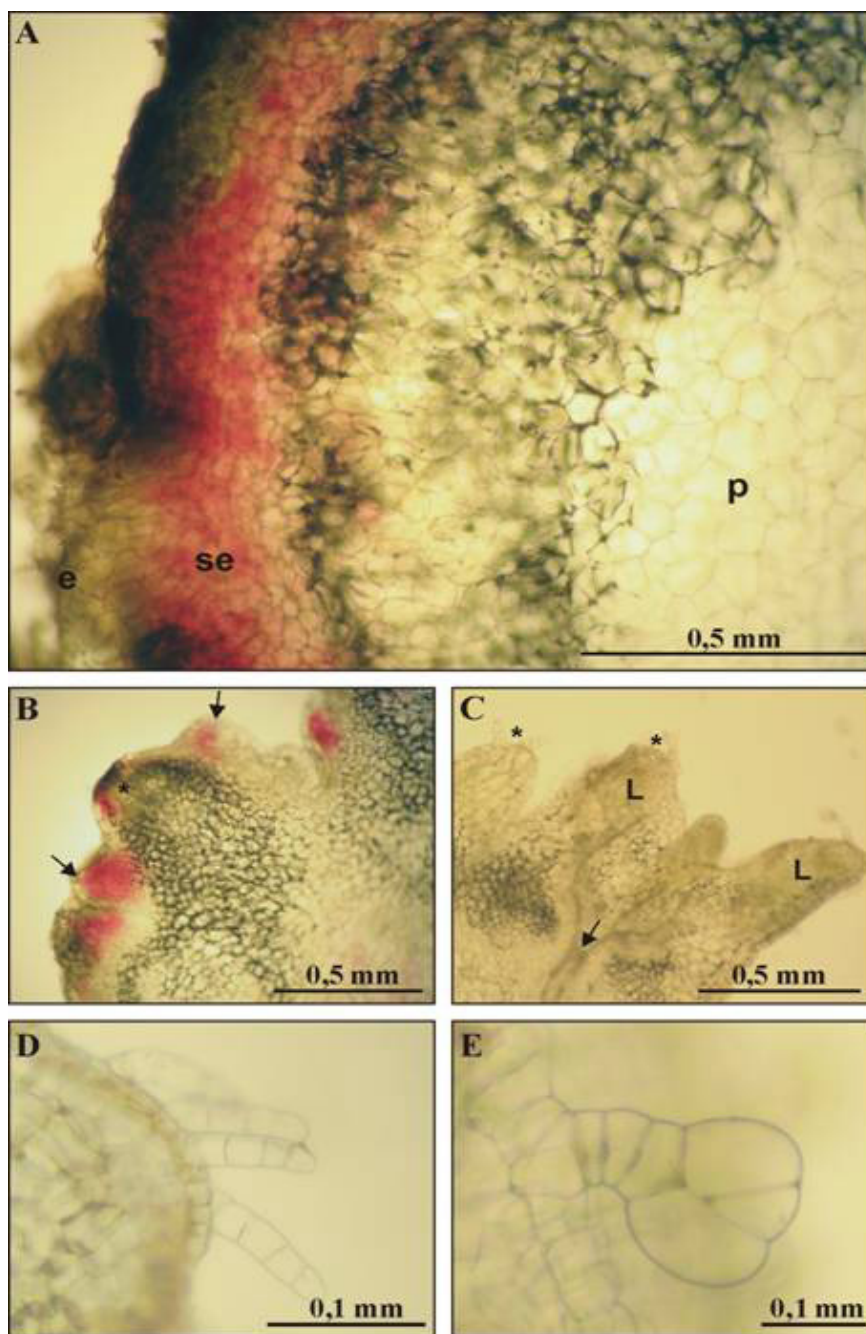


Fig. 2. Histological structure of yacon (*Smalanthus sonchifolius*) tissue similar to Protoform-Like Body (PLBs) obtained on leaves on MS medium supplemented with 1 mg L⁻¹ TDZ and 1 mg L⁻¹ NAA; e – epidermal cells, se – subepidermal cells, p – parenchymal cells, L – leaf development. A, B – red external layers of cells containing chlorophyll and centrally located, colorless parenchyma cells; B, C – organogenesis process on the different level of advancement on the surface of callus tissue; D – tissues of multicellular trichomes within the epidermis of a cover character; E – tissues of multicellular trichomes within the epidermis of a glandular character (phot. S. Glińska)

efficacy of MS-A and MS-B media was performed for the efficiency of the regeneration process.

F – Initial explants – flower buds. Regeneration was obtained from this type of initial explant on all tested media. Protocorm-like bodies obtained on leaf explants cultured on MS – M medium showed histological differentiation during anatomical studies (Fig. 2). Under the external layer of small epidermal cells (e), there was a layer of subepidermal cells (se) and in the central part the agglomeration of parenchymal cells (p). Cells with the red dye were often observed in the subepidermal layer. Within the parenchyma we could distinguished external layers of cells containing chlorophyll and also large, centrally located, colorless parenchyma cells (Fig. 2A, B). Organogenesis process on the different level of advancement was observed on the surface of callus tissue (Fig. 2B–C). In the initial stage of this process, the cluster of small meristematic cells formed the shoot apical meristem (Fig. 2B, asterisk) with the leaf development (L), the differentiation of various tissues, including vascular tissue occurred (Fig. 2C) and formation of multicellular trichomes within the epidermis (Fig. 2C, asterisks) of a cover (Fig. 2D) or glandular type (Fig. 2E).

Under the external layer of small epidermal cells, visible was a layer of subepidermal cells and in the central part the agglomeration of parenchymal cells. Shoot organogenesis was observed on the surface of the tissue (Fig. 2B–C). The cluster of small meristematic cells formed the shoot meristem with leaf primordia in the initial stage of the process (Fig. 2B).

Experiment III – rooting and acclimatization

Almost 100% of 429 rooted shoots were obtained on MS $\frac{1}{2}$ and MS $\frac{1}{4}$ media with 20 mg L⁻¹ sucrose and 1 mg L⁻¹ NAA. Rooted shoots adapted to *ex vivo* conditions in almost 100%, both in a growth chamber and greenhouse.

DISCUSSION

Estrella and Lazarte [1994], used with a success 80% ethanol and 1% calcium hypochlorite with 0.05% Tween 20 for 20 min. in order to sterilize shoots collected from jicama (*Polymnia sonchifolius*) plants grown in the field. In our experiments, similar two-step disinfection method was too drastic for initial ex-

plants, resulting in low survival and poor regeneration capacity.

Mogor et al. [2003] used buds sprouting from tuberous roots as initial explants of yacon. They disinfected them with 20% sodium hypochlorite for 20 and 40 min. However, for these explants high frequency of bacterial and fungal infections was observed. To initiate *in vitro* cultures of yacon, the various explant types have been successfully used: apical buds [Matsubara 1997], lateral buds [Estrella and Lazarte 1994, Mogor et al. 2003], shoot and root fragments [Hamada et al. 1990], nodes, leaves and their fragments [Niwa et al. 2002]. Matsubara [1997] obtained single plants from apical buds, 2 shoots from each node and callus, shoots or roots from leaf fragments. Estrella and Lazarte [1994] reported shoots development from axillary buds. All explants tested in our research demonstrated proliferation ability.

The manner and direction of shoot development depended not only on the explant type, it's structure or origin, but also on the applied medium and PGRs composition. MS medium was the most often used medium for yacon micropropagation. Paterson and Everett [1985] used MS medium for the shoot induction from callus and leaf explants, which was also applied in our experiments. Matsubara [1997] used MS medium supplemented with 0.01 mg L⁻¹ NAA and 0.01 mg L⁻¹ BA for apex buds. In our trials MS medium with the addition of the same growth substances was also applied for nodes and proved to be the most effective. Other authors applied MS medium supplemented with 2,4-D and BA in various concentrations for leaf fragments, shoots and roots. The most effective proved to be MS medium supplemented with 0.1 mg L⁻¹ 2,4-D and BA. The same medium was used by Niwa et al. [2002] for leaf fragments. Callus formation was observed most frequently on this medium. Production of callus tissue on MS medium containing 0.1 mg L⁻¹ 2,4-D and 1 mg L⁻¹ BA was reported by Matsubara [1997]. These authors observed sporadic adventitious embryos formation after transferring callus on MS medium supplemented with 0.1 mg L⁻¹ BA. Estrella and Lazarte [1994] used MS medium supplemented with IBA and BA and axillary buds to initiate *in vitro* cultures of yacon. In their studies, the most effective PGRs combination proved to be the 0.1 mg L⁻¹ IBA and 2 mg L⁻¹ BA. Niwa et al. [2002] achieved shoot organogenesis from

callus (obtained on leaf fragments) on HaR medium supplemented with 1 mg L⁻¹ BA and 0.1 mg L⁻¹ gibberellic acid (GA₃). Plants micropropagated on this medium were submitted to AFLP analysis which revealed genetic variation.

Knowing the mutagenic impact of 2,4-D on micropropagated yacon plants, described by Niwa et al. [2002], this growth regulator was excluded from our experiments. Various types of cytokinin were used for inducing regeneration from leaves. TDZ was introduced to our experiment, because there is a lot of information about its positive effects on induction of shoot regeneration and multiplication from different explants of various species, including leaves [Ernst 1994].

Singh and Dwivedi [2014] applied various cytokinins during conducting studies on multiplication of *Stevia rebaudiana*. These authors discovered that all cytokinins had an influence on shoot formation, but the most effective was TDZ. Brijwal et al. [2015] received the biggest number of shoots from callus collected from leaves of *Berberis aristata* using WP (Woody Plant) medium with the addition of 0.02 mg L⁻¹ TDZ. Siddique et al. [2015] used BA, kinetin and TDZ in order to induce shoot formation from buds during their research on micropropagation of *Cassia angustifolia*. The best results were obtained at culturing on the medium supplemented with 1 mg L⁻¹ TDZ. Ślesak et al. [2015] reported efficient shoot regeneration from root fragments of *Rumex thyrsiflorus* on MS medium supplemented with 0.5 mg L⁻¹ TDZ. Tsai et al. [2016] elaborated the method of intensive multiplication of *Salvia miltiorrhiza* from leaf explants. The largest number of shoots was obtained by direct organogenesis with the use of medium with 0.1–0.5 mg L⁻¹ TDZ. Nieves et al. [2016] obtained the greatest number of *Kalanchoe blossfeldiana* shoots from nodal explants on the medium supplemented with 0.2 mg L⁻¹ TDZ.

In our studies, the growth of a specific tissues similar to PLBs was observed when leaves and their fragments were cultured in the presence of TDZ and NAA, both at the concentration of 1 mg L⁻¹. Similar structures were described by Hong et al. [2008] for orchid *Paphiopedilum*. Kundu and Gantait [2018] showed that TDZ induced the formation of PLBs in orchid. The possibility of obtaining PLBs in *in vitro* cultures allows to elaborate a highly efficient procedure of micropropagation of yacon. Cytological observations

were conducted in order to confirm the correctness of classification of obtained cells as PLB. The comparison of the obtained image with the figure presented by Zhao et al. [2008] showed some similarity, but to confirm the presence of PLBs in Yacon, an in-depth cytological analysis should be performed.

The adventitious shoot regeneration was induced after transferring this tissue on medium containing 5 mg L⁻¹ BA and 1 mg L⁻¹ IBA. Anatomical analysis of those tissue confirmed the presence of adventitious shoot buds. In our opinion, it is a significant achievement that we managed to stimulate leaves and their fragments to generate the numerous buds by TDZ in the combination with NAA, without the use of 2,4-D. Matsubara [1997] reported that yacon shoots easily undergo the rooting process. Estrella and Lazarte [1994] obtained complete plants from axillary buds cultured on medium containing 0.02 mg L⁻¹ BA and 0.1 mg L⁻¹ IBA. Niwa et al. [2002] obtained rooted shoots on ½ MS media supplemented with 0.1 mg L⁻¹ NAA. In other studies, yacon shoots were rooted successfully on MS medium containing 0.1 mg L⁻¹ BA [Hamada et al. 1990].

Estrella and Lazarte [1994] reported that only 40% of plants adapted to *ex vitro* conditions. According to Hamada et al. [1990] yacon plants adapted easily in aired substrate under plastic tunnel within two weeks. Also, in our experiments, we discovered that yacon plants adapted quickly and easily to external conditions. Conducted studies allowed to gain the skill of controlling the process of organogenesis in yacon by using various explants and selecting proper combinations of growth regulators such as auxin and cytokinin and their concentrations. The result of this study is the elaboration of technology of intensive multiplication of yacon.

CONCLUSION

Results of conducted studies allowed to elaborate an efficient technology of multiplication of yacon in *in vitro* conditions, in which each stage of micropropagation was optimized.

The optimal explants for culture induction were shoot tips and lateral buds, from which, after the surface sterilization by method I, 1 mm long growth tips were isolated. The best for culture induction proved

to be the MS medium supplemented with 2 mg L⁻¹ kinetin, 1 mg L⁻¹ IAA and also the medium with the addition of 5 mg L⁻¹ BA and 1 mg L⁻¹ IBA. On both media, the process of callogenesis, organogenesis and also shoot multiplication occurred. On the first medium, the multiplication of small shoots was lower, but the formed shoots were ready for the rooting process, whereas on the second medium, the multiplication of small shoots was better. In the conducted experiment, beside the shoots, on these media, proliferating explants were obtained, which further multiplied the best on MS medium supplemented with 1 mg L⁻¹ TDZ and 1 mg L⁻¹ NAA. For the first time, the presence of structures similar to PLBs was discovered in *in vitro* cultures of yacon.

Shoots designated for rooting should be placed on MS ½ or ¼ media, containing 20 mg L⁻¹ sucrose and 1 mg L⁻¹ NAA, which showed 100% effectiveness of rhizogenesis.

Significance statement. Conducted studies showed that yacon is a plant with a great potential for multiplication in the tissue culture. For the first time, the presence of structures similar to PLBs was discovered in *in vitro* cultures of yacon. Obtained results are the basis for elaborating a technology to produce plant tissue in bioreactors. Thus, there will be a possibility of the wider study and usage of the bioactive substances discovered in this plant, especially the anticarcinogenic components.

ACKNOWLEDGMENTS

Research was funded by Polish Ministry of Science and Higher Education within the statutory programme No 7.4.1, 28/2010 realized by Research Institute of Horticulture in the years 2010–2014.

REFERENCES

- Brijwal, L., Pandey, A., Tamta, S. (2015). *In vitro* propagation of the endangered species *Berberis aristata* DC. via leaf-derived callus. *In Vitro Cell. Dev. Biol., Plant*, 51(6), 637–647. <https://doi.org/10.1007/s11627-015-9716-7>
- Corrêa, C.M., de Oliveira, G.N., Astarita, L.V., Santarém, E.R. (2009). Plant regeneration through somatic embryogenesis of yacon (*Smallanthus sonchifolius* Poepp. and Endl. H. Robinson). *Braz. Arch. Biol. Technol.*, 52(3), 549–554. <https://doi.org/10.1590/s1516-89132009000300005>
- Ernst, R. (1994). Effects of thidiazuron on *in vitro* propagation of *Phalaenopsis* and *Doritaenopsis* (Orchidaceae). *Plant. Cell. Tiss. Org. Cult.*, 39, 273–275. <https://doi.org/10.1007/bf00035982>
- Estrella, J.E., Lazarte, J.E., (1994). *In vitro* propagation of Jicama (*Polymnia sonchifolius* Poepp. and Endlicher): a neglected Andean crop. *Hortic. Sci.*, 29(4), 331–337. <https://doi.org/10.21273/hortsci.29.4.331>
- Hamada, M., Hosoki, T., Kusabiraki, Y. (1990). Mass-propagation of yacon (*Polymnia sonchifolia*) by repeated node culture. *Plant Tiss. Cult. Lett.*, 7(1), 35–37. <https://doi.org/10.5511/plantbiotechnology1984.7.35>
- Hong, P.-L., Chen, J.-T., Chang, W.C. (2008). Plant regeneration via protocorm-like body formation and shoot multiplication from seed-derived callus of a maudiae type slipper orchid. *Acta. Physiol. Plant.*, 30(5), 755–759. <https://doi.org/10.1007/s11738-008-0158-2>
- Kundu, S., Gantait, S. (2018). Thidiazuron-induced protocorm-like bodies in orchid: progress and prospects. In: Thidiazuron: from urea derivative to plant growth regulator. Ahmad N., Faisal M. (eds.). Springer, Singapore, 273–287. https://doi.org/10.1007/978-981-10-8004-3_13
- Lachman, J., Fernández, E.C., Orsák, M. (2003). Yacon [*Smallanthus sonchifolia* (Poepp. et Endl.) H. Robinson] chemical composition and use – a review. *Plant Soil Environ.*, 49, 283–290. <https://doi.org/10.17221/4126-pse>
- Lago, A., Godden, S.M., Bey, R., Ruegg, P.L., Leslie, K. (2011). The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. *Int. J. Dairy Sci.*, 94(9), 4441–4456. <https://doi.org/10.3168/jds.2010-4046>
- Mansilla, R., López, C., Flores, M., Espejo, R. (2010). Estudios de la biología reproductiva en cinco accesiones de *Smallanthus sonchifolius* (Poepp. & Endl.) Robinson [Reproductive biology study in five accessions of *Smallanthus sonchifolius* (Poepp. and Endl.), Robinson]. *Ecol. Appl.*, 9(2), 167–175 [in Spanish]. <https://doi.org/10.21704/rea.v9i1-2.407>
- Matsubara, S., Ohmori, Y., Takada, Y., Komadomi, T., Fukazawa, H. (1990). Vegetative propagation of yacon (*Polymnia sonchifolia*) by shoot apex, node and callus cultures. *Sci. Rep. Facult. Agri. Okayama University*, 76, 1–6.
- Matsubara, S. (1997). Micropropagation of *Polymnia sonchifolia* (Yacon). *Biotech. Agric. Forestry*, 39, 150–159. https://doi.org/10.1007/978-3-662-07774-0_10
- Millela, L., Martelli, G., Salava, J., Fernández, E., Ovesná, J., Greco, I. (2011). Total phenolic content, RAPDs, AFLPs and morphological traits for the analysis of variability in *Smallanthus sonchifolius*. *Genet. Resour. Crop. Evol.*, 58, 545–551. <https://doi.org/10.1007/s10722-010-9597-x>

- Mogor, G., Mogor, A.F., Lima, G.P.P. (2003). Bud source asepsis and benzylaminopurine (BAP) effect on yacon (*Polymnia sonchifolia*) micropropagation. *Acta Hort.*, 597, 311–314. <https://doi.org/10.17660/actahortic.2003.597.44>
- Murashige, T., Skoog, F.A. (1962). Revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15(3), 473–497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Nieves, M.C., Aspúria, E.T., Bernardo, E.C., Tayangona, M.A.D. (2016). Growth response of *in vitro*-derived nodal sections of *Kalanchoe blossfeldiana* Poellnitz as influenced by benzylaminopurine, thidiazuron and paclobutrazol. *Asia Life Sci.*, 25(1), 207–220.
- Niwa, M., Arai, T., Fujita, K., Marubachi, W., Inoue, E., Tsukihashi, T. (2002). Plant regeneration through leaf culture of yacon. *J. Japan. Soc. Hortic. Sci.*, 71(4), 561–567. <https://doi.org/10.2503/jjshs.71.561>
- Passos, L.M.L., Park, Y.K. (2003). Fructooligosacarídeos: implicações na saúde humana e utilização em alimentos [Fructooligosaccharides: implications in human health being and use in foods]. *Ciência Rur.*, 33(2), 385–390 [in Portuguese]. <https://doi.org/10.1590/s0103-84782003000200034>
- Paterson, K.E., Everett, N.P. (1985). Regeneration of *Helianthus annuus* inbred plants from callus. *Plant. Sci.*, 42(2), 125–132. [https://doi.org/10.1016/0168-9452\(85\)90152-9](https://doi.org/10.1016/0168-9452(85)90152-9)
- Siddique, I., Bukhari, N.A.W., Perveen, K., Siddiqui, I. (2015). Influence of plant growth regulators on *in vitro* shoot multiplication and plantlet formation in *Cassia angustifolia* Vahl. *Braz. Arch. Biol. Technol.*, 58(5), 686–691. <https://doi.org/10.1590/s1516-89132015050290>
- Singh, P., Dwivedi, P. (2014). Two-stage culture procedure using thidiazuron for efficient micropropagation of *Stevia rebaudiana*, an anti-diabetic medicinal herb. *Biotech.*, 4(4), 431–437. <https://doi.org/10.1007/s13205-013-0172-y>
- Ślesak, H., Góralski, G., Kwolek, D., Dziedzic, K., Grabowska-Joachimiak, A. (2015). Male adventitious roots of *Rumex thyrsiflorus* Fingerh as a source of genetically stable micropropagated plantlets. *Plant. Cell. Tiss. Org.*, 123(1), 193–203. <https://doi.org/10.1007/s11240-015-0826-z>
- Tsai, K.-L., Chen, E.G., Chen, J.-T. (2016). Thidiazuron-induced efficient propagation of *Salvia miltiorrhiza* through *in vitro* organogenesis and medicinal constituents of regenerated plants. *Acta Physiol. Plant.*, 38(1), 29–39. <https://doi.org/10.1007/s11738-015-2051-0>
- Zhao, P., Wu, F., Feng F.-S., Wang, W.-J., (2008). Prothorm-like body (PLB) formation and plant regeneration from the callus culture of *Dendrobium candidum* Wall ex Lindl. *In Vitro Cell. Dev. Biol.-Plant.*, 44, 178–185. <https://doi.org/10.1007/s11627-007-9101-2>

