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REVIEW

Polish contributions in developing medicinal plant in vitro propagation system

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Abstract

Since the 1980s, there has been a significant emphasis in biotechnology on the utilisation of medicinal plants as a source of raw materials for the pharmaceutical industry. Moreover, medicinal plants have been identified as a potential alternative source of essential compounds with a wide range of applications, including those involved in producing medications, nutraceuticals, food additives, cosmeceuticals, natural pigments, and preservatives. The plant micropropagation system is an ideal solution to the problems caused by conventional exploitation since it may simultaneously provide uniform biomass as a source of bioactive secondary metabolites and ex situ conservation of uncommon or endangered plant species (dual strategies of micropropagation). In honour of the Polish Botany Society's Centenary anniversary in 2022, this article summarises the development of an in vitro propagation method for selected medicinal plants by Polish researchers and botanists.

Key Message

This article summarises the development of an in vitro propagation and shoot cultures for selected medicinal plants by Polish researchers and botanists.

Keywords Micropropagation · Medicinal plants · Rare and protected taxa · In vitro culture · Shoot cultures

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
2iP	6-(γ,γ-Dimethylallylamino)purine
AC	Agitated culture
AS	Adenine sulfate
B_5	Gamborg medium
BAP	6-Benzylaminopurine
DIC	Dicamba
DO	Direct organogenesis
GA_3	Gibberellic acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
IO	Indirect organogenesis
KIN	Kinetin
LM	Liquid medium
LS	Linsmayer and Skoog medium
MS	Murashige and Skoog medium
NAA	1-Naphthaleneacetic acid
NN	Nitsch and Nitsch medium
NSB	Nutrient sprinkle bioreactor
PGRs	Plant growth regulators
R	Rooting
R/E	Roots per explant
RM	Reinert and Mohr medium
S	Sucrose
SH	Schenk–Hildebrandt medium
SR	Survival rate
SM	Solid medium
SS	Stationary system
SSM	Semi-solid medium
S/E	Shoots per explant
TDZ	Thidiazuron
TRIA	Triacontanol
VW	Vacin and Went medium

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WP Woody plant medium
ZEA Zeatin

Introduction

Apart from high scientific values, plant biotechnology possesses great practical importance. One of the advantages is using in vitro cultures of medicinal plants to obtain valuable secondary metabolites, which is possible thanks to the collaboration of biologists, biochemists, and technologists. The result of their study is the development of protocols for the rapid, efficient in vitro propagation of several medicinal plant species. With the simultaneous shrinkage of their natural resources, the increasing demand for medicinal plants stimulates the further development of fast reproduction methods to obtain valuable raw materials with increased biomass and a higher content of desirable secondary metabolites (Moraes et al. 2021).

Since the 1980s, medicinal plants have been of great interest in biotechnology as an essential source of raw material for the pharmaceutical industry. Nevertheless, it is estimated that only a fraction per 250,000–500,000 plant species have been scientifically investigated for their biological activity (Ngo et al. 2013). Within these species, only 15,000 of all estimated species have been documented for medicinal use (Wawrosch and Zotchev 2021). The advantages of micropropagation of various medicinal plant species have been described by numerous authors during the last four decades and reviewed in several publications (Matkowski 2008; Smetanska 2008; Moraes et al. 2021). Moreover, methods for in vitro mass propagation of selected taxa or multiplication and conservation of endangered species have been described (Murch et al. 2004; Rybczyński and Mikuła 2006; Thiem et al. 2008; Pence 2010; Tasheva and Kosturkova 2013).

The Polish Botany Society celebrates its 100th anniversary in 2022. To commemorate this occasion, we have prepared this study, which focuses on the micropropagation protocol development of chosen medicinal plants established by Polish botanists in Poland. A summary history of 65 years of plant in vitro techniques development in Poland has been described previously by Zenkteler and Zenkteler (2013). The achievements of Polish researchers in the horticultural and crop, fruits and vegetables, and ornamental plant micropropagation resulted from long-term studies in the Institute of Horticulture Research in Skierniewice (Poland) and described by Podwyszyńska et al. (2022). At the same time, reviews on Polish researchers' efforts to use in vitro culture to understand the plant developmental process at the cellular level (Płażek and Dubert 2022) and bioactive compounds production via the application of various biotechnological methods, including cell and organ cultures obtained from the cultures (Pietrosiuk et al. 2022) has been described previously.

Over the last century, many institutions and research laboratories in Poland engaged in developing micropropagation techniques for medicinal plants or plants without prior knowledge of medicinal importance. These studies have been reported within national and international scope. In this paper, we collected correlative references through the following databases: PubMed, Google Scholar, and the internal library of Poznan University of Medical Sciences (Poznan, Poland). This study aims to summarise the achievements of Polish researchers in developing in vitro propagation protocols for selected medicinal plants and potential taxa with medicinal value. A brief review of shoot cultures research, which are multiplicated as biomass for phytochemical analyses of bioactive metabolites, are also included.

Medicinal plant micropropagation system and its importance

Plants are the source of valuable substances which can be used as pharmaceuticals, nutraceuticals, food additives, cosmeceuticals, natural pigments, and preservatives. Medicinal plant species are still in great demand and play an important role in the global healthcare system. It is an essential component in herbal medicine research development. Plant secondary metabolites are utilised as a source of natural drugs (drugs of natural origin, e.g., atropine, scopolamine), raw material for semi-synthetic chemical compounds (e.g., irinotecan from camptothecin), a model for novel synthetic chemicals (e.g., cocaine for procaine), and taxonomic markers for discovering new compounds (Balunas and Kinghorn 2005; Debnath et al. 2006). Additionally, the complexity of desired products often makes it impractical and necessitates intricate multistage protocols for synthetic production. This significantly raises production costs and renders profitability uncertain. On the other hand, high demand for medicinal plants often results in habitat degradation, over-exploitation, and local species extinction because there is no control over their harvesting from the wild. The in vitro propagation system offers an integrated system of both ex situ conservation and biomass supply as a source of bioactive chemicals to resolve these concerns (Thiem et al. 2008; Tasheva and Kosturkova 2013; Moraes et al. 2021).

Some potential medicinal plants are rare, endangered or protected by the law, so their biomass is either difficult to harvest from natural habitats or inaccessible. However, these plants' bioactive compounds, often used in traditional medicine, remain unexplored. The unknown species often possess novel bioactive natural products with potential value. As an example, for the first time, the presence of iridoids and triterpenoid saponins was demonstrated in the biomass

obtained from in vitro micropropagated *Linnaea borealis*, the protected species in Poland, or phenolic compounds in clonal micropropagated *Eryngium alpinum,* endangered and protected taxa in Europe. In vitro propagation allows large biomass production without causing further damage to the naturally grown species and at the same time, allows for the initial identification of chemical profiles (e.g., *L. borealis*, *E. alpinum*, *E. maritimum*). The experiments on the rare and endangered species in Poland are tightly controlled and require special permission from the Ministry of Climate and Environment (Kikowska et al. 2014; Thiem et al. 2021).

Plant micropropagation is a process that uses in vitro technology to mass-produce valuable plant material, crop, and active ingredients. The aseptic conditions and suitable media in in vitro propagation gave more advantages than the conventional method, especially for plants with medicinal values. Traditional plant propagation is often time and labour-consuming, ineffective in many species, and does not guarantee genetic homogeneity. In vitro propagation for plants is independent of climatic factors and seasons. Therefore, it allows rapid multiplication of plants with recalcitrant or without seeds and constantly provides plant biomass. The advantages of micropropagation from various medicinal plant species have been described by numerous authors and reviewed in several publications (Bajaj et al. 1988; Rout et al. 2000; Karuppusamy 2009). The micropropagation approach also offers an integrated strategy for mass production while fulfilling the Good Laboratory Practice (GLP) and Good Manufacture Practice (GMP) to obtain better secondary metabolites output with continuous production monitoring (Matkowski 2008; Moraes et al. 2021). From the pharmaceutical point of view, the application of micropropagation methods for medicinal plants gives several benefits: acceleration of multiplication rate, uniform clones of high-yielding genotype, conservation of genetic resources, disease-free plant material, biosynthesis of novel compounds, and the identification of elite phenotypes using bioassay as a selection tool (Moraes et al. 2021).

The European Scientific Cooperation for Phytotherapy (ESCOP) commissions highlighted that the production of in vitro plant materials should be standardised and follow the GLP and GMP recommendations. Material control and quality assessment are essential throughout the process to eliminate the risk of abnormality and losing desired phytochemical properties (Purohit et al. 2011). The multiplied microplants or microshoots should undergo morphological, genetic, physiological, biochemical, and phytochemical evaluation (Fig. 1).

First, morphology assessment assures the phenotypic *true-to-type* feature of the multiplied microshoots. The

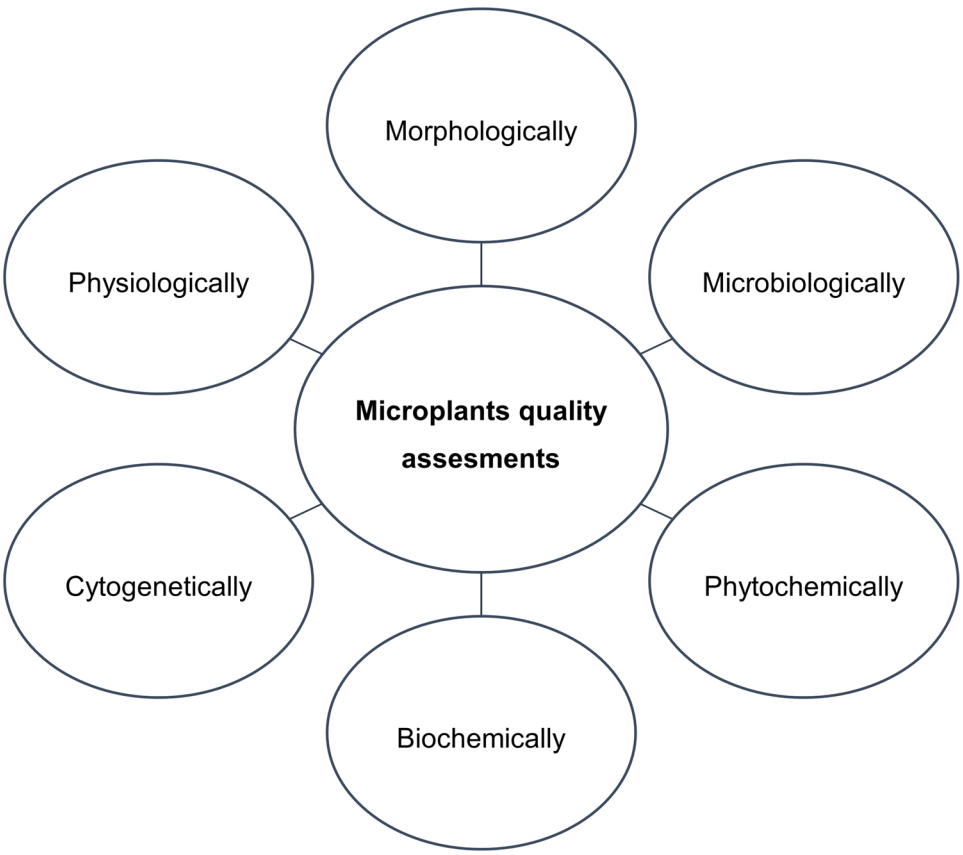

Fig. 1 Assessment parameters to obtain certificate of quality for in vitro-derived plants

interaction between the cultivated plants, cultured media composition, plant metabolism, and environmental conditions in vitro affected the plant's physiological properties. Therefore, the obtained biomass has no physiological and developmental problems, such as necrosis of the explant, hyperhydricity, fasciation, or somaclonal variations (Isah 2015). Secondly, physiology assessment evaluates the essential ability of obtained plantlets to carry out the photosynthesis process and the correct operation of the stomata. Assessment of morphology can be performed non-invasively using video cameras. The technology allows analysing stomata movement, therefore indirectly in the measurements of transpiration and photosynthesis. The results between conventional propagation and the in vitro technique could indicate the existence of differences in the structure and functions of stomata. In plants obtained from in vitro propagation, the closing of stomata in response to external stimuli is slower, which makes it necessary to maintain high humidity during acclimatisation. Photosynthesis is the physiological marker of the obtained in vitro culture. Its efficiency is assessed based on the relationship between the amount of absorbed quanta, the assimilation of one molecule of CO_2, and the production of dry mass (Bach and Pawłowska 2009). Thirdly, the genetic variations that may occur during long-term cultivation are evaluated through cytogenetic and molecular analysis. Detection of possible variations through molecular analysis can help to assure the genetic fidelity in regenerated microshoots. Flow cytometry for nuclear DNA content estimation or molecular markers detection such as restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), simple sequence repeat (SSRs), and inter simple sequence repeat (ISSR) have been applied to study and identified genetic changes in micropropagated plants (Sliwinska and Thiem 2007; Tyagi et al. 2021). Biochemical and phytochemical measurement is essential to calculate future pharmaceutical raw material devaluation. Using one- and two-dimensional thin-layer chromatography, it is possible to initially control whether the ability to biosynthesis of selected secondary metabolites is preserved in the cultivated organs and regenerated plants. Mass spectrometry techniques are resourceful in detecting changes in the metabolite production or bioactive substances of the in vitro biomass (Chun et al. 2020). Lastly, microbiological evaluation is necessary to mitigate contamination risk that can lead to spurious experimental results arising from latent contaminants or the loss of valuable experiments or commercial culture (Cassells 2012).

Recently, there has been a growing demand that plantlets produced in in vitro cultures laboratories should be certified. Propagated material for commercial purposes obtained by micropropagation should be genetically stable and pathogen-free, as documented by health certification. The certificate should include information about the high quality of the propagation product as well as the international standards-compliant manufacturing method. Such plantlets should meet the quality standard of the genotype and be in the correct physiological condition, guaranteeing adaptation to further development in vivo. Furthermore, in the case of medicinal plants, the phytochemical evaluation should demonstrate the microplants' ability to biosynthesise biologically active chemicals at a level equivalent to plants acquired using conventional procedures (van der Linde 2000).

In vitro propagation of medicinal plants in Poland

Poland is one of the prominent exporters of plant-derived medicines. According to the average amount of exported medicinal and aromatic plants between 2010 and 2019, Poland is among the top 15 exporters. This data emphasises the relevance of Polish medicinal plants in the global market (Sucholas et al. 2021).

University centres and scientific institutes have noticed the usage of plant biotechnology in the field of plant in vitro cultures. Among them are Prof. Jerzy Czosnowski and Dr. Marian Zieliński from the General Botany Department of Poznan University. They visited Roger Gautheret's plant biology laboratory and learnt about plant in vitro techniques. Upon their return, they were the first scientists applying the newly acquired knowledge in Polish laboratory practice (Zenkteler and Zenkteler 2013). Roger Gautheret was one of the three French botanists that successfully obtained unlimited growth of plant cell cultures (Thorpe 2007). Prof. Janina Rogozińska from Agricultural Academy in Bydgoszcz also visited Gautheret's laboratory and completed her post-doctoral in Skoog's laboratory. Prof. Maciej Zenkteler, after his visit to Hildebrandt and Maheshwari laboratory in Delhi, developed advanced research in experimental embryology utilising in vitro tissue techniques. For many decades he conducted practical courses and training on the plant in vitro cultures for researchers from various academic centres in Poland (Zenkteler and Zenkteler 2013).

Polish botanists who became interested in employing in vitro culture of medicinal plants included Prof. Henryk Bukowiecki and Prof. Mirosława Goleniewska-Furmanowa from the Medical University of Warsaw, along with Prof. Lutosława Skrzypczak from Poznan University of Medical Sciences. Professor Mirosława Goleniewska-Furmanowa was one of the leaders in investigating the use of medicinal plants in vitro cultures to acquire important secondary

metabolites. Her extensive practical experience, knowledge gained from foreign scientists, and unwavering research enthusiasm resulted in training many specialists in Poland and contributing to staff development in many botanical and pharmacognostic departments at universities of medical sciences (Zenkteler and Zenkteler 2013; Pietrosiuk et al. 2022).

Since the previous century, researchers have been developing micropropagation methods for medicinal plant production and in vitro propagation as a tool for producing beneficial secondary metabolites. The research was carried out at several other Polish academic institutions and research institutes, with newly emerging research centres also fulfilling the growing demand for medicinal plant micropropagation. As demonstrated in Table 1, the accomplishments of different Polish researchers in employing various plant micropropagation methods based on established protocols of selected taxa were reported in local and international journals. The monographs on methods for micropropagation of medicinal and aromatic plants and the analysis of their bioactive compounds, mainly secondary metabolites, have been published by Polish authors in several volumes of Springer-Verlag's continuous series Biotechnology in Agriculture and Forestry (Furmanowa and Olszowska 1992; Furmanowa and Rapczewska 1993; Skrzypczak et al. 1993a, b, 1996, 1998, 1999). Polish journal publishing houses, especially Acta Societatis Botanicorum Poloniae (ISSN: 0001-6977), Acta Biologica Cracoviensia Series Botanica (ISSN: 0001-5296), and Herba Polonica (ISSN: 0018-0599), also play an essential role in collecting and preserving the knowledge of micropropagation protocols for medicinal plants established by Polish researchers.

At least 265 reported studies were published in English within the Polish and English publishing houses between 1980 and 2022. Figure 2 provides an overview of the development of plant biotechnology research, especially the micropropagation of herbal plants, in Poland. The number of research significantly increases in the last decade. The Polish government, national academics, and institutes paid considerable attention to and funded research and development in this area.

Among academic institutes, there is Department of Pharmaceutical Botany and Plant Biotechnology at Poznan University of Medical Science (Poznań), Department of Biology and Pharmaceutical Botany at Medical University of Lodz (Łódź), Department of Pharmaceutical Biology and Medicinal Plant Biotechnology at Medical University of Warsaw (Warszawa), Department of Biology and Medicinal Plant Biotechnology at Medical College of Jagiellonian University (Cracow), Department of Biology and Pharmaceutical Botany at Wroclaw Medical University (Wrocław), Laboratory of Biologically Active Compounds at Intercollegiate Faculty of Biotechnology University of Gdansk and Medical University of Gdansk, Department of Pharmacognosy at Medical University of Gdansk (Gdańsk) and Department of Plant Physiology and Biotechnology at Nicolas Copernicus University (Torun). Polish research institutes engage in micropropagation of medicinal plants are the Department of Biotechnology at the Institute of Natural Fibers and Medicinal Plants (Poznań), Wroclaw Botanical Garden (Wrocław), Maj Institute of Pharmacology Polish Academy of Science (Cracow), and Polish Academy of Science in Cracow (Fig. 3).

Plant species in the field of interest of polish researchers

Polish researchers are pioneers in developing methods for selected medicinal plants' micropropagation that introduces protected or foreign species to in vitro conditions for the first time. These investigations sought novel chemicals or secondary metabolite sources with biological and/or pharmacological action. The broad spectrum of plant species belonging to various genera and families studied by Polish researchers is shown in Table 1. The choice of the studied species was mainly dictated by the possibility of obtaining raw materials for phytochemical and biological studies. The conducted research was in the scope:

1. Research and development of micropropagation strategies for obtaining new biotechnological raw materials as a potential source of crucial metabolites and for the search for new compounds with biological activity from well-studied or well-known species (e.g., *Arnica montana*, *Cannabis sativa*, *Salvia officinalis*, *Chamaenerion angustifolium*, *Plantago* spp.).
2. Biomass multiplication of protected species (e.g., *Drosera anglica*, *Eryngium maritimum*, *E. alpinum*, *Linnaea borealis*, *Primula veris*, *Rubus chamaemorus*), rare taxa (e.g., *E. campestre*, *Lychnis flos-cuculi*, *Drosera* spp.) or endemic species (e.g., *Inula verbascifolia*) for phytochemical and biological studies using the in vitro technique. These taxa's raw resources are usually unavailable because they are impossible to gather from natural sites or cannot be cultivated conventionally due to their habitat requirements.
3. In vitro studies of interesting plant species from other climatic zones (e.g., *Lithospermum canescens*, *Pueraria lobata*, *Rhaponticum carthamoides*, *Withania somnifera*, *Rehmannia glutinosa*) and search for the new biotechnological source of desired compounds.

Table 1 Micropropagation protocols via meristems, direct organogenesis (DO), and indirect organogenesis (IO) reported for selected medicinal plant species studied by Polish research groups

	Plant species (Family)	Type of explants	Methods	Best results obtained Type of media/sucrose (g/L)/ PGRs (μM)	 Yield*	Studied bioactive compounds	References
1	*Agastache rugosa* (Fischer & C.A. Meyer) (Lamiaceae)	Shoot tips	SM, SS	MS + S 30 + BAP 4.4 + IAA 0.57	69.54% SR	Volatile compounds	Zielińska et al. (2011)
2	*Arnica montana* L. (Asteraceae)	Shoot tips	SM, SS	MS + BAP 0.25 + IAA 0.5 MS	6.5 S/E 100% R High survival rate	Sesquiterpene lactones	Weremczuk-Jeżyna and Wysokińska (2000)
3	*Bergenia crassifolia* (L.) Fritsch (Saxifragaceae)	Seedling	SM, SS, IO	MS + BAP 13.2 + NAA 0.54 + AS 27.15 NN + S 40 + IBA 2.5 + KIN 0.46 + AS 27.15	SR 100% R	Arbutin	Furmanowa and Rapczewska (1993)
4	*Blackstonia perfoliata* (L.) Hudson (Gentianaceae)	Leaf	SM, SS, DO	MS + KIN 9.4 + IAA 11.4 MS + IAA 2.9	Numerous S/E Numerous R/E High survival rate	Secoiridoids (Gentiopicroside)	Skrzypczak et al. (1992, 1996)
5	*Byblis liniflora* Salisb (Byblidaceae)	Nodal	SM, SS	RM + BAP 2.2–4.4	12 S/E	Phenylethanoid glycosides (Acteosides, isoacteosides, desrhamnosylacteoside, desrhamnosylisacteoside)	Schlauher et al. (2004)
6	*Cannabis sativa* L. (Cannabaceae)	Nodal, Shoot tips	SM, SS	MS + TDZ 1.13 ½ MS + S 20 + IAA/IBA 2.9	2.5 S/E (59–70%) 1.9 R/E (74.6% R) High survival rate	Cannabinoids	Wróbel et al. (2022)
		Leaf, Stem	SM, SS, IO	MS + DIC 13.5	46 plant/E	ns	Ślusarkiewicz-Jarzina et al. (2005)
7	*Centaurium erythraea* Rafn (Gentianaceae)	Shoot tips, Seedling	SM, SS, IO	MS + BAP 4.4 + IAA 0.57 MS	43.3 S/E 94–100% R High survival rate	ns	Piątczak and Wysokińska (2003)
		Shoot tips	LM, AC	MS + BAP 4.4 + IAA 0.57 MS	60 S/E 85% R High survival rate	Secoiridoid	Piatczak et al. (2005)
		Shoot tips	SM, SS, IO	MS + BAP 4.4 + IAA 0.57 ½ MS	28 S/E 3.3 R/E High survival rate	Secoiridoid glucosides	Piątczak et al. (2011)
8	*Chaenomeles japonica* (Thunb.) Lindl. ex Spach (Rosaceae)	Axillary buds	SM, SS	MS + BAP 4.4 + IAA 0.57 MS + S 15 + IAA 5.7	5.22 S/E 3 R/E Medium survival rate	Polyphenols, Phenolic acids, Flavonoids	Kikowska et al. (2019)
9	*Chamerion angustifolium* (L.) Holub (Onagraceae)	Nodal explants	SM, SS	MS + 2iP 4.9 MS + IAA 2.9	19 S/E 9.65 R/E (98% R) High survival rate	Ellagitannins, Oenothein B, Phenolic acids	Dreger et al. (2016; 2020)
10	*Chelidonium majus* L. (Papaveraceae)	Shoot tips	SM, SS	B_5 + S 30 ½ MS + S 30 + N	10.3 S/E 18.06 R/E	Quartenary alkaloids	Zielinska et al. (2018)

Table 1 (continued)

	Plant species (Family)	Type of explants	Methods	Best results obtained Type of media/sucrose (g/L)/ PGRs (µM)	Yield*	Studied bioactive compounds	References
11	*Codonopsis pilosula* (Franch.) Nannf (Campanulaceae)	Axillary shoot	SM, SS	MS + S 20 + BAP 1 or 4 + NAA 1.0 MS + S 60 + IAA 5	38.16 S/E 7.3 R/E (98% R) High survival rate	ns	Słupski et al. (2011)
12	*Coluria geoides* Ledeb (Rosaceae)	Apical, axillary buds	SM, SS	NN + S 40 + KIN 0.46 + IBA 2.5 + AS 27.15 NN + IAA 0.57 + KIN 0.46 + 27.15	62.5% S/E 3.5 R/E (100% R High survival rate	Eugenol	Olszowska and Furmanowa (1993)
13	*Cyclopia genistoides* L. Vent (Fabaceae)	Apical shoot	SM, SS	SH + 2iP 9.84 + TDZ 1.0 ½ SH + S15 + ½ KNO_3 + ½ NH_4NO_3	8.2 S/E 54.8% R High survival rate	Xanthones, Flavonoids	Kokotkiewicz et al. (2012)
14	*Drosera aliciae* Raym.-Hamet (Droseraceae)	Leaf	SM, SS, DO	½ MS + BAP 0.4	7.5 S/E High survival rate	Ramentaceone, Naphtoquinone	Kawiak et al. (2011)
15	*Drosera anglica* Huds (Droseraceae)	Leaf, Root	SM, SS, LM, DO	Fast + S 20 + BAP 0.05 + NAA 0.005	8.3 S/E 6.4 R/E (90% R) High survival rate	ns	Kawiak et al. (2003)
16	*Drosera binata* Labill (Droseraceae)	Leaf, Shoot tips	SM, SS, LM, DO	VW + S 20	14.5 S/E (81% SR) 96% R High survival rate	ns	Kawiak et al. (2003)
17	*Drosera cuneifolia* Lf. (Droseraceae)	Leaf	SM, SS, DO	½ MS + S 20 + BAP 0.2 + NAA 0.2	6.4 S/E (25% SR) 6.4 R/E (25% R) High survival rate	ns	Kawiak et al. (2003)
18	*Drosera gigantea* L. (Droseraceae)	ns	SM, SS	½ MS + S 30 + IBA 0.05 + TDZ 1.15	34.8 S/E (72% SR)	ns	Taraszkiewicz et al. (2012)
19	*Drosera rotundifolia* L. (Droseraceae)	Leaf	SM, SS, DO	¼ MS + S 30	37.1 S/E 3.5 R/E	ns	Jadczak et al. (2017)
20	*Eryngium alpinum* L. (Apiaceae)	Axillary buds	SM, SS	MS + BAP 8.8 + IAA 5.7 + GA_3 2.9 MS + IAA 5.7 + NAA 5.4	25S/E (100% SR) 34.5 R/E (100% R) Medium survival rates	Phenolic acids, Flavonoids	Kikowska et al. (2020a)
21	*Eryngium campestre* L. (Apiaceae)	Axillary buds	SM, SS	MS + S 30 + BAP 4.4 + IAA 0.57 MS + S 50 + IAA 5.7	13 S/E (100% SR) 8.2 R/E (80% R) Medium survival rate	Phenolic acids (Rosmarinic acid and its hexoside, Chlorogenic acid)	Kikowska et al. (2016)
22	*Eryngium maritimum* L. (Apiaceae)	Axillary buds	SM, SS	MS + S 50 + BAP 4.4 + IAA 0.57 ½ MS + S 15 + IAA 0.57	4 S/E (96% SR) 22 R/E (100% R) High survival rate	Triterpenoid saponins, Phenolic acids (Rosmarinic, Chlorogenic acids)	Kikowska et al. (2014)

Table 1 (continued)

	Plant species (Family)	Type of explants	Methods	Best results obtained Type of media/sucrose (g/L)/ PGRs (µM)	Yield*	Studied bioactive compounds	References
23	*Eryngium planum* L. (Apiaceae)	Axillary buds	SM, SS	MS + S 30 + BAP 4.4 + IAA 0.57 MS + S 30 + NAA 0.54	17 S/E (100% SR) 4 R/E (90.6% R) High survival rate	Triterpenoid saponins, Phenolic acids (Rosmarinic, Chlorogenic acid, Caffeic acids)	Thiem et al. (2013)
24	*Eustoma grandiflorum* Shinn (Gentianaceae)	Shoot apices, Leaf	SM, SS	MS + KIN 9.4 + BAP 8.8 + IAA 11.4 MS + IAA 2.9	c.a. 25 S/E 90% R	Gentiopicrosides	Skrzypczak et al. (1993a)
25	*Genista tinctoria* L. (Fabaceae)	Shoot tips	SM, SS	SH + IAA 5.7 + 2iP 9.84 + TDZ 0.99 SH + NAA 2.68	30.8 S/E (91% SR) 13.2 R/E (100% R)	Genistein-7-*O*-diglucoside, 2′-hydroxy genistein7-*O*-diglucoside, Daidzin, Apigenin-7-*O*-diglucoside, Luteolin-7-*O*-diglucoside, Genistin, Genistin malonate, Genistin acetate, Daidzein, Genisten, Luteolin, Sissotrin	Łuczkiewicz et al. (2005)
26	*Harpagophytum procumbens* (Burch.) DC. ex Meisn (Pedaliaceae)	Shoot tips	SM, SS	SH + TDZ 6.0 MS + IAA 0.57	11.2 S/E (96.7% SR) 4.6 R/E (90% R) Low survival rate	ns	Grąbkowska and Wysokińska (2009)
		Shoot tips	SM, SS	SH + S 30 + BAP 8 + IAA 0.57 SH + S 30 + IAA 0.57	7 S/E 3.77 R/E (86.7% R) High survival rate	ns	Grąbkowska et al. (2014)
27	*Inula verbascifolia* ssp. *aschersoniana* (Asteraceae)	Shoot tips, Axillary buds	SM, SS	MS + BAP 2.22 + NAA 0.26 MS + IBA 0.98	7 S/E 100% R	Parthenolide	Thiem et al. (2003)
28	*Inula royleana* DC (Asteraceae)	Cotyledonary node, Axillary buds	SM, SS	MS + NAA 0.1 + KIN 5.0 ½ MS	5.1 S/E (94% SR) 70% R	ns	Stojakowska and Malarz (2004)
29	*Linnaea borealis* L. var. *borealis* (Linnaeaceae)	Shoot tips, Shoot clusters	SS, LM	MS + BAP 4.4 + IAA 5.7 + GA_3 2.9 MS + IAA 5.7 + IBA 9.8	17 S/E 19 R/E (100%R) Low survival rate	Phenolic acids, Flavonoids, Iridoids, Triterpenoid saponins	Thiem et al. (2021)
30	*Lithospermum canescens* (Michx.) Lehm (Boraginaceae)	Shoot tips, Stem fragments	SM, SS	LS + BAP 8.8 + KIN 2.3 ½ B_5 + IBA 0.98	8.22 S/E 46% R High survival rate	ns	Sykłowska-Baranek et al. (2004)
31	*Lychnis flos-cuculi* L. (Caryophyllaceae)	Shoot tips, Axillary buds	SM, SS	MS + S 30 + BAP 4.4 + IAA 0.57 MS + S 30 + IAA 0.57	16 S/E Rooting High survival rate	Phytoecdysteroids (20-hydroxyecdysone, Polypodine B)	Maliński et al. (2019)

Table 1 (continued)

	Plant species (Family)	Type of explants	Methods	Best results obtained Type of media/sucrose (g/L)/ PGRs (μM)	 Yield*	Studied bioactive compounds	References
33	*Lycopus lucidus* Turcz ex Bent (Lamiaceae)	Shoot tips	A	MS	100% SRSR 90% R High survival rate	ns	Zielińska et al. (2006)
34	*Oenothera biennis* L. (Onagraceae)	Shoot tips, Axilarry buds	SM, SS	MS + BAP 4.4 + IAA 0.57 or NAA 5.4 MS + IBA 2.5	4–10 S/E Root regeneration	Fatty acids in seeds, Palmitic, Stearic, Oleic, Linoleic, γ-Linolenic acids	Skrzypczak et al. (1994)
35	*Oenothera paradoxa* Hudziok (Onagraceae)	Shoot tips, Nodal segments, Axilarry buds	SM, SS	MS + BAP 4.4 + IAA 5.7 MS + IAA 5.7 or IBA 4.9	12 S/E 100% R	Fatty acids in seeds of in vitro propagated plants, γ-linolenic acid	Skrzypczak et al. (1998); Thiem et al. (1999)
36	*Onosma paniculatum* (Bur. and Franch) (Boraginaceae)	Shoot tips, Stem segments	SM, SS	LS + BAP 8.8 + KIN 2.3 LS	3.0 S/E 36.7% R	ns	Sykłowska-Baranek et al. (2004)
37	*Primula veris* L. (Primulaceae)	Shoot tips	SM, SS	MS + BAP 4.44 + 2,4D 1.13 MS + IBA 2.45	5.9 S/E 100% R High survival rate	Flavonoids	Morozowska and Wesołowska (2004)
38	*Plantago asiatica* L. (Plantaginaceae)	Shoot tips	SM, SS	MS + BAP 4.4 + IAA 0.57 MS	5.2 S/E R	ns	Makowczyńska and Andrzejewska-Golec (2003)
39	*Plantago camtschatica* Link (Plantaginaceae)	Shoot tips	SM, SS	MS + BAP 8.8 + IAA 0.57 MS + NAA 2.7	7.2 buds and S/E 8.1 R/E	ns	Andrzejewska-Golec and Makowczyńska (2008)
40	*Plantago lanceolata* L. (Plantaginaceae)	Leaf, Root	SM, SS, DO	MS + KIN 9.29 + IAA 11.42 MS + IAA 5.71	4.4 S/E 100% R High survival rate	Phenylethanoid glucosides	Budzianowska et al. (2004)
41	*Plantago maritima* L. (Plantaginaceae)	Shoot tips	SM, SS	MS + BAP 2.2 MS + NAA 0.5	8.8 buds and S/E 89% R High survival rate	ns	Makowczyńska and Andrzejewska-Golec (2009)
42	*Plantago media* L. (Plantaginaceae)	Shoot tips, Seedling explants	SM, SS, DO, IO	MS + BAP 8.8 + IAA 5.7 MS + IAA 5.7	9.2 S/E 100% R High survival rate	Phenylethanoids (Acteoside, Plantamajoside)	Budzianowska et al. (2019)
43	*Pueraria lobata* (Willd.) Ohwi (Leguminosae)	Shoot tips	SM, SS	MS + S 30 + KIN 4.6 + IAA 5.7 MS + S 30 + IAA 11.42	3.6 S/E (94% SR) 10.9 R/E (100% R) High survival rate	Isoflavonoids (Formononetin, Daidzein, Genistein)	Thiem (2003)
44	*Rehmannia glutinosa* Libosch (Scrophulariaceae)	Shoot tips	SM, SS, NSB	MS + BAP 4.4 + IAA 0.57 MS + IBA 0.49	8.2 S/E (SM/SS) 21 S/E (NSB) 5.3 R/E (93% R) High survival rate	Phenolics Flavonoids	Piątczak et al. (2014)

Table 1 (continued)

	Plant species (Family)	Type of explants	Methods	Best results obtained Type of media/sucrose (g/L)/ PGRs (μM)	Yield*	Studied bioactive compounds	References
45	*Rhaponticum carthamoides* (Willd.) Iljin (Asteracaeae)	Leaf	SM, SS, DO, IO	MS + S 30 + BAP 2.2 + NAA 1.08 ½ MS + S 30 + IBA 4.92	4.5 S/E (100% SR) 4.3 R/E (88% R) High survival rate	Chlorogenic acid and 20-hydroxyecdysone	Skała et al. (2015)
46	*Rhodiola kirilowii* Rgl. ex Maxim (Crassulaceae)	Axillary buds	IO, A	MS	100% plantlets regeneration	ns	Zych et al. (2005)
47	*Rhododendron tomentosum* Harmaja (Ericaceae)	Nodal segments, Axillary buds	SM, SS	SH + S 30 + TDZ 1.0 + 2iP 9.84 SH + S 30 + 2iP 9.84 ½ WP + S 10 + IBA 4.92	276.3 growth index 107.8 S/E c.a. 10 R/E (100% R) High survival rate	Essential oils	Jesionek et al. (2016)
48	*Rubus chamaemorus* L. (Rosaceae)	Shoot tips, Axillary buds	SM, SS	MS + BAP 0.88–8.8 + IBA 0.49–2.45 ½ MS + IBA 98	12 S/E (> 95% SR) 5 R/E (58% R) High survival rate	ns	Thiem (2001)
49	*Salvia nemorosa* L. (Lamiaceae)	Shoot tips, Leaf	SM, SS	MS + S 30 + BAP 4.4 + IAA 2.9 MS + S 30 + NAA 0.5	5.7 S/E (shoot tips) 6.6 S/E (DO) 3.7 R/E (100% R) High survival rate	ns	Skała and Wysokińska (2004)
50	*Salvia officinalis* L. (Lamiaceae)	Shoot tips	LM, SS, AC	MS + S 30 + TRIA 0.05 + BAP 2.22 + IAA 0.57 ½ MS + S 30 + IAA 0.57	6.7 S/E 35% R High survival rate	Diterpenoids (carnosic acid, carnosol), rosmarinic acid, total phenolic content	Grzegorczyk and Wysokińska (2008)
		Shoot tips	SM, SS	MS + S 30 + BAP 8.0 + IAA 0.57 ½ MS + S 30	3 S/E (73.5% SR) 3.7 R/E (73.8% R) High survival rate	ns	Grzegorczyk and Wysokińska (2004)
51	*Salvia przewalskii* Maxim (Lamiaceae)	Shoot tips	SM, SS	MS + TDZ 4.5 + IAA 0.57 MS + IBA 0.5	3.2 S/E Rooting High survival rate	Essential oils	Skała et al. (2007)
52	*Salvia sclarea* L. (Lamiaceae)	Shoot tips	SM, SS	MS + S 30 + BAP 4.4 + IAA 0.57 MS	3.6 S/E 90% R High survival rate	ns	Skała and Wysokińska (2001)
53	*Salvia splendens* Kerr. Gawl (Lamiaceae)	Shoot tips	SM, SS	MS + S 30 + BAP 4.4 + IAA 0.57 LS	4.3 S/E 72% R High survival rate	ns	Skała and Wysokińska (2001)

Table 1 (continued)

	Plant species (Family)	Type of explants	Methods	Best results obtained Type of media/sucrose (g/L)/ PGRs (μM)	Yield*	Studied bioactive compounds	References
54	*Scutellaria alpina* L. (Lamiaceae)	Shoot tips	SM, SS	MS + S 30 + BAP 2.2 IAA 0.57	25 S/E	Baicalin, Wogonoside, Luteolin, Luteolin-7-*O*-glucoside, Verbascoside	Grzegorczyk-Karolak et al. (2015a)
			LM, SM/ SSM, SS, NSB	MS + S 30 + IAA 0.57 + BAP 2.22 (LM) ½ MS (SM)	36.1 S/E 4 R/E (92% R) High survival rate	Baicalin, Wogonoside, Luteolin, Luteolin-7-*O*-glucoside, Verbascoside	Grzegorczyk-Karolak et al. (2017b)
55	*Scutellaria baicalensis* Georgi (Lamiaceae)	Nodal fragments	SM, SS	MS + S30 + NAA 0.5 + KIN 2.5 ½ MS + S30	3.8 S/E (70% SR) Rooting	ns	Stojakowska et al. (1999)
56	*Solidago virgaurea* L., *S. canadensis* L., *S. gigantea* Ait (Asteraceae)	Axillary shoots	SM, SS	MS + S30 + KIN 9.3 + IAA 11.4 MS + IAA 5.7	Rooting High survival rate	Essential oils	Kalemba and Thiem (2004)
57	*Stevia rebaudiana* Bert (*Asteraceae*)	Shoot tips	SM, SS	MS + BAP 4.4 + GA_3 0.29 MS + IBA 4.9	11.3 S/E 10.4 R/E	ns	Doliński and Jabłonska (2015
58	*Teucrium scorodonia* L. ssp. *scorodonia* (Lamiaceae)	Shoot tips, Nodal segments	SM, SS	MS + S30 + BAP 8.8 or ZEA 9.2 + IAA 0.57 MS + S30 + IAA 2.9	7/8 S/E 13 R/E (100% R) High survival rate	Essential oils	Makowczyńska et al. (2016)
59	*Thymus vulgaris* L. (Lamiaceae)	Apical and axillary buds, Nodal segments	SM, SS	NN + S 20 + KIN 0.46 + NAA 0.54 NN + S20 + IBA 2.5 + AS 27.15 NN + S 40 + IBA 2.5 + AS 27.15	Shoot multiplication 90% R 5 R/E (90% R) High survival rate	Essential oil	Furmanowa and Olszowska (1992)
60	*Urginea maritima* (L.) Baker (Lamiaceae)	Bulb scales, Leaf	SM, SS	MS + BAP 8.8 + NAA 10.8	25 S/E	ns	Stojakowska (1992)
61	*Withania somnifera* (L.) Dun (Solanaceae)	Shoot tips, Axillary buds	SM, SS	NN + BAP 4.4 + IBA 4.9 NN + KIN 0.46 + IBA 2.5 + AS 27.15	120 S/E 90% R High survival rate	Withanolides (Withaferin A)	Furmanowa et al. (2001)

2,4-D 2,4-dichlorophenoxyacetic acid, *2iP* 6-(γ,γ-dimethylallylamino)purine, *A* alginate encapsulation, *AC* agitated culture, *AS* adenine sulfate, B_5 Gamborg medium, *BAP* 6-benzylaminopurine, *DIC* dicamba, *DO* direct organogenesis, *E* explant; GA_3 gibberellic acid, *IAA* indole-3-acetic acid, *IBA* indole-3-butyric acid, *IO* indirect organogenesis, *KIN* kinetin, *LM* liquid medium, *LS* Linsmayer and Skoog medium, *MS* Murashige and Skoog medium,, *NAA* 1-naphthaleneacetic acid, *NN* Nitsch and Nitsch medium, *NS* not specified, *NSB* nutrient sprinkle bioreactor, *PGRs* plant growth regulators, *R* rooting, *R/E* roots per explant, *RM* Reinert and Mohr medium, *S* sucrose, *SH* Schenk-Hildebrandt medium, *SM* solid medium (c.a. 7 g/L agar), *SS* stationary system, *SR* shoot regeneration, *SSM* semi solid medium (c.a 3.5 g/L agar), *S/E* shoots per explant, *TDZ* thidiazuron, *TRIA* triacontanol, *VW* Vacin and Went medium, *WP* woody plant medium, *ZEA* zeatin

*Survival rate: 0–40% low; 41–70% medium; > 70% high

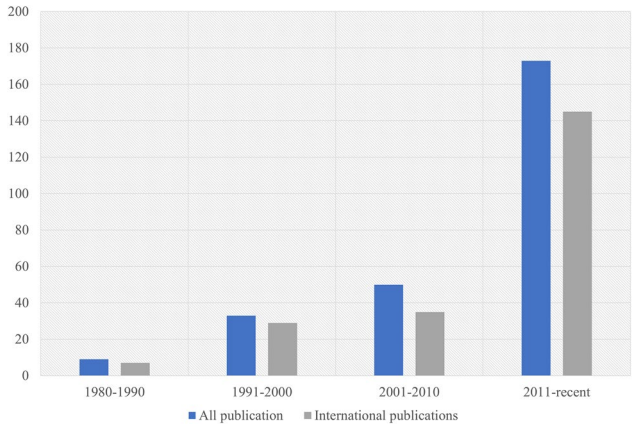

Fig. 2 Journals and chapters published by Polish researchers between 1980 and 2023 on the topic of medicinal plant in vitro cultures. The number of publications were collected from three national publishing house (Acta Societatis Botanicorum Poloniae, Acta Biologica Cracoviensia Series Botanica, and Herba Polonica), and two international publishers (Springer and Elsevier)

The developed micropropagation protocols

Micropropagation protocol development requires selecting appropriate plant material and developing methods for the multiplication of shoots and the induction and development of roots. Then further develop these whole plantlets to be ready for acclimatisation. Several parameters require optimisation throughout this process, such as: choosing the suitable media type, improving with hormonal supplementation (combination and concentration of plant growth regulators), and finding a suitable culture system. Explants applied in the multiplication process were selected after analysing the biology of the species and the availability of plant material.

Primary explants selection

Propagation of medicinal plants has been achieved in in vitro culture, usually through the proliferation of small explants such as shoot tips and axillary buds. High-quality raw materials of micropropagated plants with stable properties could be obtained via in vitro technique by collecting primary explants isolated from naturally-grown plants without causing damage to the ecosystem. Moreover, propagation from existing meristem parts yields genetically and biochemically identical multiplied plantlets to the donor plants, which is desirable and beneficial from a pharmaceutical point of view (Bajaj et al. 1988; Thiem and Kikowska 2008).

The primary explants for initiation of in vitro culture were often part of whole plants or seeds that aseptically germinated, young parts of in vitro-derived plantlets. The explants with meristematic tissue, such as shoot tips (e.g., *Arnica montana*, *Plantago* spp., *Salvia* spp., *Pueraria lobata*), axillary buds (*Codonopsis pilosula*, *Eryngium* spp., *Solidago virgaurea*), and nodal explants (e.g., *Chamaenerion angustifolium*, *Thymus vulgaris*), were chosen in these studies as listed in Table 1. During the subculture, shoots were multiplied via the natural development of the existing meristematic parts, unexpanded leaves at different development stages, and several primordia (Bhatia and Sharma 2015). Plantlet production in micropropagation could undergo direct organogenesis from any plant tissue or indirect organogenesis via dedifferentiated cells (Bhatia and Sharma 2015). Several studies covered in this review paper achieved plant regeneration indirectly from callus (e.g., *Bergenia crassifolia*, *Cannabis sativa*, *Centaurium*

Fig. 3 Several well-known research centers in Poland, which are studying the medicinal plant micropropagation

erythraea, *Plantago media*, *Rhaponticum carthamoides*), most often occurring on seedling or leaf explants. The direct organogenesis method was less often used to perform shoot regeneration and multiplication (e.g., *Blackstonia perfoliata*, *Drosera* spp., *Rhaponticum carthamoides*).

Media selection

The most widely used basic medium in the process of plant micropropagation was Murashige and Skoog (MS) and modifications thereof, such as half-strength MS (½ MS). MS basal media has the most prosperous macronutrient composition among all mentioned basic media, making good plant regeneration. However, the MS medium was not the best choice for optimum development in certain circumstances since it had a high ammonium-to-nitrate ratio (Phillips and Garda 2019). Thus, other media, such as Gamborg (B_5), Nitsch and Nitsch (NN), Linsmaier and Skoog (LS), Schenk and Hildebrandt (SH), or Reinert and Mohr (RM) were used for the selected medicinal plant (Table 1).

Ammonium levels are lower in both B_5 and LS media, making it the best medium for propagating *Lithospermum canescens*. The Nitsch and Nitsch (NN) media has approximately half of MS's salts composition but composes of a similar ammonium-to-nitrate ratio which is ideal for shoot multiplication in *Bergenia crassifolia*, *Coluria geoides*, *Thymus vulgaris*, and *Withania somnifera*. Woody plant medium (WPM), which has lesser amounts of macronutrient salt, was used for *Rhododendron tomentosum* clonal propagation following its woody characteristics. In rare cases, the Fast media or Vacin and Went (VW) media were used for *Drosera anglica* and *D. binata* and Reinert and Mohr (RM) media for the propagation of *Byblis liniflora* (Table 1). The Fast and VW media are generally macro- and micronutrient deficient compared to others and addressed for the orchid. However, Kawiak et al. (2003) proved that these media, along with the half-strength MS, were preferred for *Drosera* micropropagation. Other media originally intended for orchids were also suitable for *Drosera* based on the protocol developed by Kukułczanka and Czastka (1988).

Studies of *Drosera* spp. have been aimed at determining the most favourable media conditions for their growth (Kukułczanka and Cząstka 1991; Kawiak et al. 2003). There seems to be no universal medium for all Droseraceae species. A study by Kawiak et al. (2003) identified MS medium with mineral content reduced by half as the one most effective for some *Drosera* species. Królicka et al. (2008) claimed that the best medium for *Drosera binata* propagation was Vacin and Went. Jadczak et al. (2017) indicated that the medium most favorable for the growth of *D. rotundifolia* was ¼ MS. The plants grown on this medium had the greatest weight and produced the most significant number of progeny plants. The number of plantlets cultured on different media—1/8 MS or ½ MS was significantly lower. The study of Jadczak (2017) also showed a clear correlation between reduced content of minerals and increased number of roots. The greatest number of long roots was developed on ¼ MS medium. Numerous roots were also developed by shoots cultures on the media with the content of nitrogen compounds reduced by half (Jadczak et al. 2017).

Plant growth regulators (PGRs)

The balance of plant growth regulators (endogenous synthesised and exogenous applied) guarantees success at every stage of micropropagation. The types and amounts of growth regulators utilised in these experiments differed between species and were chosen with the biology and physiology of a particular taxon in mind.

In light of research confirming the usefulness of PGRs, cytokinins level especially is critical for the multiplication of shoots. The 6-benzyladenine (BAP) is the first generation of synthetic cytokinins that are often added to the media individually or in combination with auxin in these studies and significantly caused the formation and development of shoots, e.g., *Centaurium erythraea* (44.4 S/E), *Codonopsis pilosula* (38.2 S/E), *Linnaea borealis* (17 S/E), *Scutellaria alpina* (36.1 S/E), and *Withania somnifera* (120 S/E).

Thidiazuron (TDZ) is a urea-derivative cytokinin with a dynamic role in plant tissue culture. TDZ has been applied in in vitro techniques to induce adventitious shoot formation or promote axillary shoot proliferation (Lu 1993). The action of TDZ is highly dependent upon its concentration, exposure time, and cultured explants (Ahmad and Shahzad 2018). The addition of TDZ to *Cannabis sativa* caused phenotypic vitrification, leaf narrowing, leaf rolling, and suppressed the growth of shoots, which are highly undesirable in biomass production (Wróbel et al. 2022). Jesionek et al. (2016) reported that a combination of TDZ and 6-(γ,γ-Dimethylallylamino)purin (2iP) on the endangered essential oil-bearing *Rhododendron tomentosum* efficiently produced a massive number of shoot primordia at the multiplications stage (276.3 growth index) after 30 days of cultivation. The morphology of the microshoots was highly affected by the presence of both cytokinins. The microshoots were healthy and without any signs of necrosis. However, they were short and formed calluses at the base of explants. In the study of *Harpagophytum procumbens* conducted by Grąbkowska et al. (2014), pretreating the explants with TDZ for 6 h and then cultured to SH medium containing 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA) increased the shoot multiplication up to 330% compared to non-pretreated explants. Nevertheless, it caused dwarfism and hyperhydricity of the microshoots. On the other hand, *H. procumbens* microplants pretreated with TDZ had higher survival ability and morphological features than non-pretreatment plants

(Grąbkowska and Wysokińska 2009; Grąbkowska et al. 2014).

Besides cytokinin supplementation, adenine sulphate and gibberellic acid were also added to the media. Adenine sulphate (AS) acts as a precursor for natural cytokinin synthesis or enhances the natural synthesis of cytokinin. In this way, it enhances the growth and proliferation of axillary shoots and promotes adventitious shoot formation (George et al. 2008). Better shoot development was observed on media with AS in *Bergenia crassifolia*, *Coluria geoides*, *Rubus chamaemorus*, *Thymus vulgaris*, and *Withania somnifera* (Table 1) supplementation. Excessively high adenine sulphate concentration could additionally induce organogenesis. As a result, shoots formed from the meristematic tissue and adventitious arise via organogenesis (e.g., *B. crassifolia*). Gibberellic acid (GA_3) is simple natural gibberellin and has been applied to culture media to promote rapid stem elongation, an essential step before rooting (Phillips and Garda 2019). Thus, it became a choice for shoot elongation by some researchers (e.g., *Eryngium alpinum*). The GA_3 supplementation to media was the most suitable media for long-term culture and effective proliferation (Kikowska et al. 2020a, b).

Among various auxins applied for in vitro rooting, the natural auxin IAA is the most commonly used in the selected studies, such as *Coluria geoides* (100% R), *Eustoma grandiflorum* (90% R), *Plantago lanceolata* (100% R), and *Pueraria lobata* (100% R). Synthetic auxins, indole-3-butyric acid (IBA; e.g., *Coluria geoides* (100%R), *Inula verbascifolia* (100% R), and *Rehmannia glutinosa* (93% R), α-naphthaleneacetic acid (NAA; e.g., *Drosera anglica* (90%), *D. cuneifolia* (25%), *Plantago maritima* (89% R), and *Salvia nemorosa* (100% R)) were also used in several studies. Thiem (2003) observed the impact of NAA, IBA, and IAA supplementation in various concentrations on root induction of *P. lobata*. From the result, IAA promoted root induction more effectively (100% R). Nevertheless, plantlets rooted on medium with IAA grew slowly and became yellow. Meanwhile, plantlets grown on NAA had thicker and shorter roots with callus-forming capacity, which reduced the capability of plantlets to survive during acclimatisation. A combination of natural and synthetic auxin, such as IAA and NAA on *Eryngium alpinum*, promoted rooting up to 34.5 R/E. The supplementation of both auxins significantly improved rooting compared to in vitro rhizogenesis occurring in the case of single auxins (2.9 and 7.5 R/E on IAA and IBA, respectively). The presence of NAA in the medium induced relatively short, thick, dark roots. The generally shallow structure allows root to quickly obtain water before it evaporates. Extensive branching of those roots may extend the area of obtaining water with mineral salts, which is important in the process of planting the plant to ex vitro conditions. From a pharmaceutical point of view, the fibrous system is also more attractive because most often noticeable is higher production of desired secondary metabolites. Young roots and lateral roots are more suitable for the production of bioactive compounds (Kikowska et al. 2020a, b). Similar roots' morphology was also observed in *E. planum*. The addition of IBA to the media generated lateral roots on the microplants. Meanwhile, IAA supplementation resulted in long, thin roots with fewer lateral roots. The initially whitish roots subsequently turned dark brown and decreased in length along with shoot maturation, indicating an increased secondary metabolite concentration (Thiem et al. 2013). Some plants may undergo spontaneous rooting on full-strength or half-strength media without plant growth regulators (e.g., *Arnica montana*, *Centaurium erythraea*, *Lycopus lucidus*, and *Scutellaria alpina*). Efficient root formation on auxin-free media may occur due to high endogenous auxin availability in in vitro shoots. Therefore, exogenous addition on media is not essential to induce root formation (Piątczak and Wysokińska 2003).

As previously mentioned, genetic instability may arise as the period of in vitro propagation increases and as constant exposure to a high level of plant regulators. The genetic stability assessment of in vitro propagated plants should be carried out each time while developing the protocols. This evaluation is essential to monitor and maintain the level of metabolites from the raw material. Detection based on a morphological feature only is undependable and imprecise, as phenotypically similar explants may possess different molecular properties. In order to confirm the genetic fidelity of plantlets, cytogenetic (flow cytometry) or molecular methods, such as amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR), or randomly amplified polymorphic DNA (RAPD), were often applied (Sliwinska 2018).

In vitro environmental stress

Environmental stress is essential in determining plant structure and function in closed and open environments. Furthermore, it is also the key determinant for fluctuation in plant secondary metabolites (Verma and Shukla 2015). Adamczuk et al. (2012) reviewed the possible role of stress and reactive oxygen species in morphogenic process of in vitro plants. In this controled micropropagation system, osmotic stress induced by high or low sucrose content of the media, reduction in the mineral composition of the media, high concentration of exogenous plant growth regulators, high and low temperature, quality of illumination, propagation system including agitated tissue and organ cultures could trigger explants' response to stress, varied on the species. In *Codonopsis pilosula*, for example, high sucrose concentration in media affected the morphology of the shoots and roots to become thicker and

shorter (Słupski et al. 2011). The study of osmotic stress on the endangered *Rhododendron tomentosum*, presented that along the the increasing of sucrose concentration, root induction is also enhanced (Jesionek et al. 2016).

The use of the agitated culture system with total immersion was not recommended in the case of *Salvia officinalis* L. and *Eryngium alpinum* L. cultivation. Micropropagated shoots obtained by this method displayed malformations and undesirable physiological abnormalities. In these cases, it was probably an improper ratio of the volume of the medium to the culture vessel (Grzegorczyk and Wysokińska 2008; Kikowska et al. 2020a). The shoots of propagated *Linnaea borealis* L. immersed in higher volume of the medium in RITA bioreactor (200 ml in comparison to 150, 100 or 80 ml) were characterised by overgrowth and hyperhydricity (Kikowska et al. 2022). The stationary liquid culture for shoot multiplication of *Scutellaria alpina* L. was supported by polyurethane foam (PUL system) or bacterial nanocellulose (BNCL system). The concentrations of bioactive metabolites, including baicalin, verbascoside and flavones in the liquid cultures (BNCL, PUL) were significantly higher than those in shoots cultured on agar solidified medium (Grzegorczyk-Karolak et al. 2017b). The introduction of *Genista* species into in vitro culture conditions negatively affected the flavones production but improved the quantity of isoflavones (Łuczkiewicz and Piotrowski 2005). Their results suggested that different propagation conditions impacted the secondary metabolite production.

Propagation systems: stationary, agitated, and bioreactor

The propagation is carried on solid or liquid media stationary or agitated system, respectively (Fig. 3). The number of new shoots multiplied in liquid media was usually more significant than on solid media, as shown in the study of *Drosera anglica*, *D. binata*, *Linnaea borealis*, *Salvia officinalis*, *Scutellaria alpina*, *Lychnis flos-cuculi* (Table 1). The liquid system allows explants to have a larger surface area and faster nutrient uptake, supported by constant agitation. The application of liquid system to micropropagate *D. anglica* and *D. binata* significantly improved the proliferation rate up to twofold than in solid system (Kawiak et al. 2003). However, the fluid system is not favourable for several species due to the appearance of hyperhydricity, an unusual apoplastic water accumulation resulting in morphology abnormalities (e.g., thickened stems, short internodes, fragile and twisted leaves). Adverse effects of the liquid culture system for in vitro plants were shown in the study of *D. cuneifolia* (Kawiak et al. 2003) and *S. alpina* (Grzegorczyk-Karolak et al. 2017b). The continuous contact between plants and media might cause cultures to become necrotic and fail to proliferate. The problem was then overcome by using cellulose bridges to support the shoot cultures, allowing partial contact between plant material and liquid medium. This method improved the multiplication rate of *S. alpina* compared to other tested systems with very low hyperhydricity events (Grzegorczyk-Karolak et al. 2017a, b). Another solution to overcome these issues is by selecting the appropriate volume of liquid media for the size of culture vessels. As the explants are not completely immersed in the medium, vitrification of plant tissue can be avoided, as conducted by Kikowska et al. (2022) on *Linnaea borealis* shoot cultures.

The culture containers were mainly using Erlenmeyer flasks. Nutrient sprinkle bioreactors and temporary immersion systems were also utilised to increase the size of the culture and boost micropropagation productivity. Shoots proliferation of *Rehmannia glutinosa* was significantly increased when grown in a nutrient sprinkle bioreactor (NSB; 21 S/E) than in a stationary system (8.2 S/E) (Piątczak et al. 2014). The shoot cultures of *L. borealis* also grew efficiently in a temporary immersion system RITA® bioreactor up to 894% of fresh weight biomass ratio (Kikowska et al. 2022). However, biomass production using bioreactors does not constantly improve the yield, as observed in the study of *Scutellaria alpina* where the shoot multiplication rate only increased slightly compared to the solidified system (Grzegorczyk-Karolak et al. 2017b). Therefore, further optimisations of the bioreactor's protocols for each species are highly needed.

Acclimatisation

The success of the micropropagation process depends on the plantlets' transfer to ex vitro conditions as the final step. Multiplied whole plants are transferred into acclimatisation in order to produce plants for outplanting or reintroduction to natural habitat. Microplants transferred to ex vitro conditions are exposed to abiotic (altered temperature, light intensity, and humidity) along with biotic stress from soil microflora.

Proper rooting of the explant is crucial to ensure the plant's survivability. In the study of *Codonopsis pilosula*, the author emphasised that different rooting systems obtained from in vitro propagation influenced survival rates. Roots with shorter and thicker characteristics had a higher survival rate as they could cope with soil conditions (Słupski et al. 2011). A high percentage of rooting correlates with high survival acclimatisation. Plantlets with high rooting chances had a higher survival rate, as observed in several species with (*Rhaponticum carthamoides*—direct organogenesis (88% R)) compared to a low rooting percentage (e.g., *R. carthamoides*—indirect organogenesis (59% R)) (Skała et al. 2015). The difference in humidity between in vitro (high

humidity) and ex vitro (low humidity) conditions could induces abiotic stress to the plants during acclimatisation. Retardation of cuticle development, epicuticular waxes and functional stomatal apparatus could occur during in vitro culture and causes high transpiration rates of plantlets ex vitro (Chandra et al. 2010). Therefore, the authors often covered the plantlets with glass or plastic to ensure high humidity (e.g., *Rehmannia glutinosa*, *Centaurium erythraea*, and *Lychnis flos-cuculi*) or adjusted the light intensity by acclimatising to the dark tunnel (e.g. *Chamaenerion angustifolium*). Maliński et al. (2019) further observed the 98% survival rate of *L. flos-cuculi* from three batched of 25 plants in the experimental plot for two vegetation systems from the previous 91% survival frequency during acclimatisation in pots.

Micropropagated plants, after transfer to soil (ex vitro) conditions are often characterised by better morphology parameters (such as a higher number of branched shoots) and physiology conditions (e.g., more vigour), which then correlate with a higher biomass of the potential pharmaceutical raw materials. For example, in the second year of vegetation, the seeds of propagated *Oenothera biennis* and *O. paradoxa* plants had a higher content of oil and γ-linolenic acid than those from intact plants (Skrzypczak et al. 1994; Thiem et al. 1999). Similar results were also seen in the in vitro-derived *Lychnis flos-cucuili* plants, where biomass quality and quantity improved. More branched shoots with inflorescences were observed, and the sum of main ecdysteroids (20-hydroxyecdysone and polypodine B) from the flowering herb ex vitro plants was higher than the flowering herb of intact plants (Malinski et al. 2019). Kikowska et al. (2018) reported that the sum of six main saponins significantly increased in the root vigor system of in vitro-derived *Eryngium planum* plants grown in an experimental plot up to 7 times more naturally grown plants.

Chemical potential of in vitro propagated medicinal plants

Developing a micropropagation system is the first step in providing an alternative biomass supply for quantifying or isolating active chemicals of interest to the pharmaceutical sector. Biomass extraction from regenerated plants cultivated on a laboratory scale and in various experimental plots is part of the strategy for introducing these species into cultivation. Phenolic acids, flavonoids, phenylethanoids, triterpenoids, saponins, ecdysteroids, and essential oils were among the substances studied in phytochemical investigations of many medicinally important plant species. Several studies have revealed an increase in the production of specific secondary metabolites in the organs of micropropagated plants compared to intact plants, which Polish researchers have accomplished.

Apiaceae

The genus *Eryngium* (*E. alpinum*, *E. campestre*, *E. maritimum*, and *E. planum*) is part of the Saniculoideae subfamily from the family Apiaceae, and the metabolites composition was extensively studied by Kikowska et al. (2014; 2016; 2020b) and Thiem et al. (2013). *Eryngium* is rich in phenolic acids, flavonoids (mostly kaempferol and quercetin derivatives), triterpenoid saponins derivatives of acylated R1 and A1-barrigenols, and essential oils. These metabolites were effectively produced using in vitro culture systems and biotechnology techniques. The content of selected phenolic acids was reported to be higher in shoots and roots collected from micropropagated *Eryngium* species than from analogous organs of soil-grown plants. The content of the sum of phenolic acids in leaves from in vitro-derived plantlets was 1.67 higher from *E. planum* and 6.75 from *E. maritimum* than in the rosette leaves of the plants from natural sites (Thiem et al. 2013; Kikowska et al. 2014). Interesting research focuses on producing triterpenoid saponins, important compounds with promising anticancer properties, in the tissues of micropropagated *E. planum* plantlets. The shoots produced 8.7 times more sum of five main triterpenoid saponins than leaves of field-grown plants, and roots of micropropagated plantlets accumulated 3.3 times more than roots of field-grown plants (Kikowska et al. 2018). Essential oils obtained from *E. planum* rosette leaves of field-grown plants and shoots from in vitro propagated plantlets had different compositions. The in vitro multiplied shoots of *E. planum* are an outstanding source of (*Z*)-falcarinol (64.4% of essential oil), the most bioactive compounds in the falcarinol–type polyacetylenes (Thiem et al. 2011). On the other hand, the in vitro-derived shoots of *E. alpinum* plantlets were characterised by essential oil composed mainly of hexadecanoic acid (15.5%), spathulenol (7.5%), (*E*)-β-farnesene (4.9%), germacra-4(15),5,10(14)-trien-1α-ol (4.7%), and falcarinol (4.3%) (Kikowska et al. 2020b). The pronounced amounts of some sesquiterpenes in the shoot oil of *E. planum*, namely hydrocarbons with eremophilane and selinane skeletonm (*E*)-nerolidol, and two ketones β-elemenone and germacrone (Thiem et al. 2011).

Asteraceae

The Asteraceae family grows in diverse habitats and is one of the most prominent flowering plant families. The medicinal effect of members from the Asteraceae family comes from its phytochemicals, such as inulin, polyphenols, phenolic acids, flavonoids, acetylenes, sesquiterpene lactones,

and triterpenes. These compounds provide antioxidant, anti-inflammatory, antimicrobial, and antiplatelet activities. Moreover, the Asteraceae family possess prebiotic, wound healing, diuretic and hepatoprotective properties (Rolnik and Olas 2021). Valuable phytochemicals, such as sesquiterpene, lactone, parthenolide, and essential oils, were successfully obtained from the in vitro biomass of *Arnica montana*, *Inula verbacifolia*, and *Solidago* spp., respectively (Weremczuk-Jeżyna and Wysokińska 2000; Thiem et al. 2003; Kalemba and Thiem 2004).

The essential oils obtained from four *Solidago* species (*S. virgaurea*, *S. canadensis*, *S. gigantea*, and *S. graminifolia*) proliferated in in vitro cultures produced the same yield and similar compositions as plants from the natural sites. The primary essential oil compound in in vitro propagated *S. canadensis* and *S. virgaurea* was α-pinene (59.5 and 25.4%, respectively), a constituent with fresh pine scent and woody flavour, and limonene (97 and 14.8%, respectively). The α-pinene plays a crucial role in the fragrance and flavour industry. α-pinene's biological characteristics include antibacterial, antifungal, anti-leishmania, anti-inflammatory, anti-oxidative, antiapoptotic, neuroprotective, and gastroprotective effects (Allenspach and Steuer 2021). Among the studied species, germacrene D, a common sesquiterpene with insecticidal and repellent activities, was one of the major constituents in *S. canadensis*, *S. graminifolia*, and *S. gigantea* (15.2%, 12.1%, and 12.8%, respectively) (Kalemba and Thiem 2004). Thus, in vitro propagated plants of *Solidago* species could be a source of bioactive compounds, especially α-pinene, for pharmaceutical, fragrance, and flavor applications.

Stevia rebaudiana Bert. has intense sweet taste, which it owes to specific diterpene glycosides. The greatest influence on the quality of the raw material and level of sweetness are stevioside and rebaudoside A. As a herbal medicine, *S. rebaudiana* is a valuable source to fight microbial pathogens, good sweetener source for diabetic or hypoglycemic patients, high-blood pressure patient, and for people with obesity. The pharmaceutical industry uses glycosides to improve the taste of bitter tablet and syrups, and to produce basic components to protect teeth from caries (Doliński and Jabłonska 2015).

Droseraceae

An interesting subject of long-term interdisciplinary studies based on the collaboration of several institutions to search for new compounds with therapeutic activity is carnivorous plants from the Droseraceae family. The first research on *Droseraceae* was carried out in Wroclaw Botanical Garden using *D. intermedia*, *D. rotundifolia*, *D. spatulata*, and *D. bredifolia*. The laboratory of Plant Protection and Biotechnology of the Intercollegiate Faculty of Biotechnology in Gdańsk had optimised the media and in vitro culture conditions of *D. anglica*, *D. binata*, *D. cuneifolia*, *D. capensis*, *D. aliciae*. The in vitro culture allows the increase in the propagation rate of important compounds, such as naphthoquinones, flavonoids, antocyanins, and phenolic compounds. Banasiuk et al. 2012 had reviewed the application of in vitro culture for secondary metabolites production. The biomass of plants belonging to the *Drosera* genus was successfully obtained thanks to the in vitro culture technique. Biomass is the source for isolating and characterising selected naphthoquinones e.g., rossoliside (7-methylhydrojuglone 4-*O*-glucoside) and hydroplumbagin 4-*O*-glucoside from micropropagated plantlets of *D. rotundifolia* and *D. intermedia*, respectively (Budzianowski et al. 1993; 1996).

Gentianaceae

Plants from the family Gentianaceae are known for their ornamental features, yet upon the discoveries of secondary products from this family the research of these plants for their medicinal values stimulated. In Poland, the research on Gentians were boosted after the publication of the 'Red Book'of Polish Flora (Rybczyński et al 2015). The family Gentianaceae has xanthones, secoiridoids, and flanovoids as their phytochemical characteristics (Skrzypczak et al. 1993a, b). The gentiopicroside (secoiridoid) content in *Blackstonia perfoliata* L. was dominant about 3.55 times higher in shoot of micropropagated plantlets compared to the ground plants (Skrzypczak et al. 1992). The presence of flavonoids in *B. perfoliata* in vitro shoot culture was also confirmed using the two-dimensional thin layer chromatography (2D TLC) on polyamide (Skrzypczak et al. 1996). Both gentiopicroside and flavonoids were also observed in *Eustoma grandiflorum* Shinn using thin layer chromatography (Skrzypczak et al. 1993a, b). *Centaurium erythraea* Rafn is used as herbal medicine (*Centaurii herba*) for its therapeutic effects as blood and kidney cleanser, indigestion treatment, healing wound and reducing sores. It is later known that secoiridoid glucosides (gentiopicroside, swertiamarin, and sweroside) are main components in these medical values of *C. erythraea*. The biomass of in vitro shoots of *C. erythraea* contained significantly higher gentiopicroside and swertiamarin, but no significant difference on sweroside content compared to wild-grown plants. Interestingly, the total secoiridoid contents were 1.9-threefold higher in shoots from micropropagated plantlets than in wild-grown plants (Piączak et al. 2005).

Lamiaceae

Lamiaceae family species are rich in essential oils, therefore, valuable in the cosmetic, flavoring, fragrance, perfumery, and pharmaceutical industries. They are often grown as culinary herbs for edible leaves. Lamiaceae's most prominent

secondary metabolites are phenols (tannins, quinones, flavonoids, lignans, and terpenoids) (Carović-Stanko et al. 2016). The essential oils of Lamiaceae were studied in the biomass of micropropagated *Teucrium scorodonia* (Makowczyńska et al. 2016) and *Thymus vulgaris* (Furmanowa and Olszowska 1992). The chemical composition of essential oils from plants and in vitro-derived was similar and characterised by high level of sesuiterpene hydrocarbons, such as β-caryophyllene, germacrene, and α-humulene. The results indicate that in vitro-derived plants maybe a potential source of essential oil with β-caryophyllene as the main component, approved as food additive. A simple procefure for rapid propagation of *T. vulgaris* was applied for obtaining of selected genotypes with different biological and chemical charaters, but the essential oils composition was similar and thymol, a compound with strong antimicrobial properties, was identified as the main compound (Furmanowa and Olszowska 1992). In whole shoots of *Salvia officinalis* micropropagated plants grown in greenhouse, the content of diterpenoid were different than in commercially available dried leaves (1.2–1.37 higher). Biotechnologically obtained biomass can be an important source of rosmarinic acid, a polyphenol constituent of many herbs for food and beverage applications, as a dietary supplement, pharmacy, and cosmetics. From their studies, the rosmarinic acid content from the whole shoots was 1.67–9.33 higher than the commercial dried leaves (Grzegorczyk and Wysokińska 2008). Extensive studies were conducted on in vitro propagation of *Scutellaria alpina* and its secondary metabolites production (Grzegorczyk-Karolak et al. 2015a ; 2017b). According to their findings, in vitro stationary shoots in liquid media of *S. alpina* are an excellent source for producing bioactive substances with diverse pharmacological properties, a flavone glucuronide (baicalin), flavonoid (wogonoside), and phenylethanoid (verbacoside), with the highest content of predominant metabolite baicalin (c.a. 18.79 mg/g) (Grzegorczyk-Karolak et al. 2015a). The scale-up of in vitro *S. alpina* shoot cultures using the bioreactor slightly increased the biomass production (Grzegorczyk-Karolak et al. 2017b). The essential oil composition of micropropagated shoots of *Agastache rugosa* appeared to be different than adult plant. The biomass of in vitro-derived shoots was rich in α-pinene, limonene, isomethone, and pulegone. While the adult plant possesses mostly estragole, a natural organic compound, which has been used as additive, flavoring agents, and fragrance, that were absent in the in vitro shoot cultures (Zielińska et al. 2011). Essential oils composition in in vitro shoot cultures was also studied in *Salvia przewalskii* by Skała et al. (2007). The highest essential oil constituents found in leaves of in vitro cultures were limonene and β-phellandrene, β-caryophyllene, and bornyl acetate. The micropropagated leaves consisted of higher bornyl acetate, a camphoraceous oil, (up to 3.9 times) and β-caryophyllene, a natural bicyclic sesquiterpene, (2.3 times) compared to the leaves from in vivo plants (Skała et al. 2007).

Plantaginaceae

Species from the family Plantaginaceae are also one of the most studied taxa by Polish researchers, especially from the genus *Plantago*. Makowczyńska and Andrzejewska-Golec described the micropropagation protocol of *P. asiatica* (2003), *P. camtschatica* (2008), and *P. maritima* (2009). Meanwhile, *P. lanceolata* and *P. media* were studied by Budzianowska et al. (2004, 2019). Early analysis found that iridoids are the chemotaxonomic markers of the genus *Plantago* (Andrzejewska-Golec et al. 1993). Further research of the *Plantago* species, the composition of iridoids and organ-specific phenylethanoid glucosides (e.g., acteoside and plantamajoside) were identified and measured in *Plantago.* Quantitative screening of the extract using TLC video densitometric showed a significant improvement in the accumulation of acteoside in shoots (93.03 mg/g dry weight) and plantamajoside in roots (44.08 mg/g) of in vitro propagated *P. media.* Phenylethanoids in the leaves of micropropagated plantlets, compared to soil-grown plants estimated by other authors. As the authors stated, the organ of in vitro propagated plants may be considered an alternative source of pharmacologically active phenylethanoids (Budzianowska et al. 2019). In the shoots of micropropagated *P. lanceolata*, acteoside and plantamajoside were 17.85 and 0.56 mg/g, respectively. *P. lanceolata* regenerated in vitro had a phenylethanoid composition similar to plants from natural sites, except for minor compounds (martynoside in place of isoacteoside) (Budzianowska et al. 2004).

Onagraceae

Several species of *Oenothera* have been known for their medicinal oils, which are assessed for cosmetic industries. A typical evening primrose in Poland, *O. biennis*, gains medicinal importance through the presence of γ-linoleic acid in the seeds. Skrzypczak et al. (1994) compared the oil and fatty acids content in *O. biennis* of seeds from in vitro-derived plants and ground cultivation. From their study, plant regeneration using the biotechnology technique slightly improved the total oil content (24%) and fatty acid contents, including γ-linoleic acid (8.2%), in comparison to intact plants (23 and 7.7%, respectively). Thiem et al. (1999) also observed that the content of γ-linoleic acid in the seeds of in vitro propagated *O. ammophila* and *O. paradoxa* after the first season of vegetation was comparable with those from intact plants. Oenothein B, a cyclic dimeric ellagitannin, is present in many medicinal plants and has been reported to have a wide range of biological

Table 2 Biotechnological approaches on shoot cultures of selected medicinal plants and cultures conditions optimisation for biomass and bioactive compounds production studied by Polish research groups

No	Species (Family)	Experimental works	Studied secondary metabolite	References
1	*Agastache rugosa* (Fisch. & C.A.Mey.) Kuntze (Lamiaceae)	Shoot tips, solid media, LED illumination and amino acid supplementation	Phenolic acids (Rosmarinic acid)	Zielińska et al. (2019)
2	*Aronia melanocarpa* (Michx.) Elliott, *Aronia arbutifolia* L., *Aronia*×*prunifolia* (Marsh.) (Rosaceae)	Leaf buds, solid media, impact of monochromatic lights	Phenolic acid, Depsides, Flavonoids	Szopa et al. (2014, 2018b)
3	*Centella asiatica* (L.) Urban (Apiaceae)	Tops of shoots, solid media, liquid media, bioreactor Plantform and RITA bioreactor, agitated culture, precursor (L-Phe) and elicitor supplementation (MeJa and ETH)	Centellosides, Flavonoids, Phenolic acids	Skrzypczak-Pietraszek et al. (2019)
4	*Dionaea muscipula* J. Ellis (Droseraceae)	Solid media, elicitation using JA, nitrogen deficiency, lysate of *Agrobacterium rhizogenes*	Naphthoquinones, Flavonoids	Królicka et al. (2008)
		Solid media, blue-red light elicitation	Phenolic compounds	Makowski et al. (2019)
		Solid and liquid agitated media, biotic elicitation and hydromechanical stress	Myricetin, Caffeic acid, Ellagic acid and Plumbagin	Makowski et al. (2020)
5	*Dracocephalum forrestii* W. W. Smith (Lamiaceae)	Shoot tips, solid media, cytokinins impact	Rosmarinic acid, Salvianolic acid B	Weremczuk-Jeżyna et al. (2018)
		Nodal explants, liquid media, NSB bioreactor	Caffeic acid derivatives (Rosmarinic acid, Salvianolic acid B)	Weremczuk-Jeżyna et al. (2019a)
		Solid and liquid media, *A. rhizogenes* transformation, cytokinins impact	Rosmarinic acid, Flavonoid derivatives	Weremczuk-Jeżyna et al. (2019b)
		Nodal segments, liquid media, *A. rhizogenes* transformation, bioreactors (RITA, Plantform, and NSB)	Rosmarinic acid, Acacetin	Weremczuk-Jeżyna et al. (2020)
		Nodal segments, solid media, *A. rhizogenes* transformation, LED light treatment	Acacetin, Apigenin glucosides	Weremczuk-Jeżyna et al. (2021b)
		Nodal segments, solid media, LED light treatment	Rosmarinic acid, Salvianolic acid B, Apigenin derivatives	Weremczuk-Jeżyna et al. (2021a)
		Nodal segments, liquid media, *A. rhizogenes* transformation, RITA bioreactor	Acacetin rhamnosyl-trihexoside, Rosmarinic acid, Apigenin glucoside	Weremczuk-Jeżyna et al. (2023)
6	*Drosera* sp. (Droseraceae)	Solid media, elicitation using JA, nitrogen deficiency, lysate of *A. rhizogenes*	Naphthoquinones, Flavonoids	Królicka et al. (2008)
		Solid media, blue-red light elicitation	Phenolic compounds	Makowski et al. (2019)
7	*Exacum affine* Balf. f. ex Rege (Gentianaceae)	Stem fragments, solid media, liquid agitated media, callus-derived shoots, elicitation, precursor feeding (L-Phe, MeJa), sucrose impact	Phenolic acids	Skrzypczak-Pietraszek et al. (2014)
8	*Hypericum perforatum* L. cultivars: 'Elixir', 'Helos' and 'Topas' (Hypericeae)	Solid media	Indole compounds: 5-hydroxy-L-tryptophan, Hypericin, Serotonin,	Muszyńska et al. (2014)
		Liquid media, agitated shoot culture	Flavonoids	Kwiecień et al. (2018)
		Solid and liquid media, agitated, bioreactor and precursor (L-Phe) feeding	Total phenolic, Flavonoids, Tannin	Kwiecień et al. (2023b)

Table 2 (continued)

No	Species (Family)	Experimental works	Studied secondary metabolite	References
9	*Leucojum aestivum* L. (Amaryllidaceae)	Solid media, liquid media, RITA bioreactor, indirect somatic embryogenesis	Alkaloids (Galanthamine, Lycorine)	Ptak et al. (2012)
10	*Melittis melissophyllum* L. (Lamiaceae)	Seedlings, solid media, liquid agitated media, precursor feeding and elicitor supplementation (L-Phe, ETH, MeJa)	Iridoid glycosides (Harpagide, 8-*O*-acetyl-harpagide)	Skrzypczak-Pietraszek et al. (2018b)
11	*Nasturtium officinale* R. Br (Brassicaceae)	Agitated, liquid media, effect of metal salts	Glucosinolates, Phenolic acids	Klimek-Szczykutowicz et al. (2019)
		Solid, agitated liquid media, PGRs impact	Phenolic acids	Klimek-Szczykutowicz et al. (2020b)
		Liquid media, RITA bioreactor	Glucosinolates, total Phenolics, total Flavonoids	Klimek-Szczykutowicz et al. (2020a)
		Plantform bioreactor, precursor (L-Phe, Tryp) feeding	Total Glucosinolates, total Flavonoids, total Polyphenols, selected Polyphenols (*p*-coumaric acid, Ferulic acid, Rutoside)	Klimek-Szczykutowicz et al. (2021)
		Agitated liquid media, elicitation (MeJa, SS, YE)	Total Glucosinolates, total Flavonoids, total Polyphenols	Klimek-Szczykutowicz et al. (2022)
12	*Polyscias filicifolia* (C.Moore ex E.Fourn.) L.H.Bailey (Araliaceae)	Solid media, elicitation (MeJa, SA, ADT)	Total Phenolic acids, total Flavonoids, Oleanolic acid	Sliwińska et al. (2021)
13	*Pontechium maculatum* L. Böhle & Hilger (Boraginaceae)	Solid media, liquid media, Plantform bioreactor	Total Phenolics, selected Phenolic acids and Flavonoids	Makowski et al. (2023)
14	*Rehmannia elata* N.E.Br. ex Prain (Orobanchaceae)	Apical meristems, solid media, light and cytokinin treatment	Iridoid glycoside (Harpagide), Phenylethanoid glycosides (Verbascoside, Isoverbascoside)	Piątczak et al. (2020)
15	*Ruta chalepensis* L., *Ruta Corsica* DC, *Ruta graveolens* L. (Rutaceae)	Agitated liquid media	Selected linear Furanocoumarins and Furoquinoline alkaloids	Szewczyk et al. (2022)
16	*Ruta graveolens* L. (Rutaceae)	Stationary liquid system	Total Furanocoumarins, Umbelliferone	Ekiert et al. (2001)
17	*Ruta graveolens* L.	Agitated liquid media	Total Furanocoumarins, Umbelliferone	Ekiert and Czygan (2005)
	Ruta graveolens ssp. *divaricata* (Tenore) Gams (Rutaceae)	Stationary liquid system, light treatments	Phenolic acids, total Furanocoumarins, Umbelliferone	Szopa et al. (2012)
18	*Ruta graveolens* ssp. *divaricata* (Tenore) Gams (Rutaceae)	Stationary liquid system, callus-derived shoots	*P*-coumaric acid and other phenolic acids	Ekiert et al. (2014)
19	*Salvia sclarea* L. (Lamiaceae)	Shoot tips, solid media	Essential oils	Kuźma et al. (2009)
20	*Salvia viridis* L. (Lamiaceae)	Shoot tips, solid media	Phenolic acids (Rosmarinic acid), Phenylethanoids	Grzegorczyk-Karolak et al. (2019)
		Shoot tips, solid media, impact of cytokinins	Phenolic acids, Phenylethanoids	Grzegorczyk-Karolak et al. (2020)
		Liquid media, Plantform bioreactor	Phenolic acids, Phenylethanoids	Grzegorczyk-Karolak et al. (2022)

Table 2 (continued)

No	Species (Family)	Experimental works	Studied secondary metabolite	References
21	*Salvia bulleyana* L. (Lamiaceae)	Shoot tips, solid media, clonal propagation and organogenesis	Polyphenol compounds	Grzegorczyk-Karolak et al. (2021)
		Shoot tips and nodal segments, solid media, impact of cytokinin	Phenolic acids	Grzegorczyk-Karolak et al. (2023)
22	*Schisandra chinensis* (Turcz.) Baill (Schisandraceae)	Leaf buds, solid media, shoot-differentiating callus cultures	Phenolic acids and Lignans	Szopa and Ekiert (2011, 2012)
		Solid media and liquid media, stationary and agitated cultures	Dibenzocyclooctadiene lignans	Szopa et al. (2016)
		Solid media, shoot-differentiating callus cultures from leaf buds, light treatment	Dibenzocyclooctadiene lignans	Szopa and Ekiert (2016)
		Liquid media, 5 bioreactor types	Dibenzocyclooctadiene lignans	Szopa et al. (2017)
		Liquid media, Plantform bioreactor, elicitation ($CdCl_2$, Ch, YE, MeJa)	Dibenzocyclooctadiene lignans	Szopa et al. (2018a)
		Solid media, liquid media, agitated, Plantform bioreactor	Phenolic acids and Flavonoids	Szopa et al. (2019)
23	*Schisandra rubiflora* Rehd. et Wils (Schisandraceae)	Liquid media, agitated, Plantform bioreactor, elicitation (ETH, YE, Ch, MeJa)	Lignans	Szopa et al. (2022)
24	*Scutellaria alpina* (Lamiaceae)	Shoot tips, solid media	Flavonoids (Baicalin, Wogonoside, Luteolin), Phenylethanoid glycoside (Verbascoside)	Grzegorczyk-Karolak et al. (2016)
25	*Scutellaria altisima* L. (Lamiaceae)	Shoot tips, solid media	Baicalin, Wogonoside, Luteolin, Luteolin-7-*O*-glucoside, Verbascoside	Grzegorczyk-Karolak et al. (2015b)
		Shoot tips, solid media, impact of cytokinins	Phenolic compounds	Grzegorczyk-Karolak et al. (2017a)
26	*Scutellaria brevibracteata* (Rech.f.) Greuter & Burdet (Lamiaceae)	Shoot tips, solid media	Flavonoids, Phenylpropanoid glycosides, Phenolic acids	Kwiecień et al. (2023a)
27	*Scutellaria lateriflora* L. (Lamiaceae)	Shoot tips, solid media, impact of different light conditions	Baicalin, Verbascoside	Kawka et al. (2017)
		Seedlings, solid media, liquid media, agitated system, Plantform bioreactor, precursor (L-Phe and Tyr) and elicitation (MeJa)	Flavonoids, Verbascoside	Kwiecień et al. (2022)
28	*Vitex agnus castus* L. (Lamiaceae)	Shoot fragments, solid media, liquid agitated media, precursor (L-Phe)	Flavonoids and Phenolic acids	Skrzypczak-Pietraszek et al. (2018a)

ADT alarmone-diadenosine triphosphate, *$CdCl_2$* cadmium chloride, *Ch* chitosan, *ETH* ethephon, *JA* jasmonic acid, *L-Phe* L-phenylalanine, *MeJa* methyl jasmonate, *NSB* nutrient sprinkle bioreactor, *PGRs* plant growth regulators, *SA* salicylic acid, *SS* sodium salicylate, *Tryp* tryptophan, *Tyr* tyrosine, *YE* yeast extract

activities (antioxidant, anti-inflammatory, anti-viral, antifungal, and antitumor). *Chamaenerion angustifolium* is one of the medicinal plants that produce oenothein B. The shoots of acclimatised *C. angustifolium* plantlets were shown to have 1.85 higher contents of oenothein B than the shoots of cultivated plants (Dreger et al. 2020). Dreger et al. (2022) also analysed the content of sterol in *C. angustifolium* in vitro plant cultures, and they concluded that micropropagated *C. angustifolium* cultures are a rich source of stigmasterol (375.64–577.77 mg/100 g dry weight) and campesterol (375.64–577.77 mg/100 g dry weight), which were 66.14 and 9.17 times higher, respectively, compared to the field-grown *C. angustifolium* plants. Both campesterol and stigmasterol have anti-inflammatory and anticarcinogenic activity.

Shoot cultures as biomass for production of important bioactive compounds

Table 2 summarizes the accomplishments of Polish scientists in utilizing shoot cultures at different developmental stages (shoot-differentiated callus, microshoots, shoot culture) within various in vitro systems (stationary, agitated, temporary immersion, liquid-phase, gas-phase). These cultures were grown on different substrates (agar-solidified, liquid) and supplemented with diverse phytohormones to serve as multiplied biomass for phytochemical research. These studies aimed to assess the ability of shoot cultures under different physicochemical conditions to biosynthesize species-specific metabolites and determine their potential as an alternative source of bioactive compounds. In order to optimize the growth of biomass and multiplication of shoots as well as the production of selected metabolites, shoot cultures were grown under variable in vitro conditions and subjected to various biotechnological treatments. These treatments included the use of elicitors, new regulators of plant growth and development (BAP derivatives), different lighting conditions (LED, monochromatic, UV lights), and cultivation in bioreactors (NSB, RITA®, and Plantform™). The qualitative and quantitative analyses focused on phenolic acids, flavonoids, phenylethanoids, lignans, iridoids, furanocoumarins, and alkaloids, revealing the ability of shoot cultures to synthesize these metabolites. Comparative studies often demonstrated higher efficiency of shoot cultures in producing the tested bioactive compounds compared to corresponding organs from natural plants, leading to the development of robust culture protocols.

Conclusion

The in vitro culture method is used to propagate medicinal plants in controlled cultivation conditions, even on a larger scale. Micropropagation techniques have been used for medicinal plants whenever vegetative reproduction was necessary to obtain homogenous plant material with phytochemical characteristics identical to the donor plant. As this method involved only organised meristems, hence it allows to obtain genetically stable and true-to-type microplants. Using in vitro technologies as dual strategy for ex situ conservation and a source of bioactive compounds of rare and protected species could be realised. The Polish researchers have already contributed to developing micropropagation protocols for these medicinally valuable plants. The pioneering studies started in the 1980s, and the legacy is still progressing until this time. Thanks to the multidisciplinary collaboration, it is possible to obtain biomass for phytochemical and biological research. Hopefully, the efforts will keep improving and lead to the successful production of biomass and valuable active compounds that benefit society.

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Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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