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Potential applications of plant *in vitro* cultures in phytoremediation studies

Marta Jaskulak[1], Anna Grobelak[1]
1Czestochowa University of Technology,
Faculty of Infrastructure and Environment,
J.H. Dąbrowskiego 69, 42-201 Częstochowa
e-mail: martajaskulak@gmail.com

The main aim of this review is to assess the advantages and disadvantages of use of in vitro plant cell and organ cultures as useful research tools in process of phytoremediation. Plant tissue cultures including cell suspensions, callus and hairy roots are frequently used in the phytoremediation research, mostly as a model plant systems. One of the most important advantages of using in vitro cultures is the ability to examine the metabolic capabilities of plant cells as well as their capacity for toxicity tolerance in controlled conditions without any interference from microorganisms and processes occurring naturally in soils. The results obtained from plant cell or tissue cultures can be used to predict the responses of plants to environmental stressors and also to mass produce stress induced proteins and other metabolites. The aim of this review is to present possible applications for in vitro cultures in phytoremediation studies.

Keywords: phytoremediation, heavy metals, in vitro cultures, soil contamination

Introduction

Rapid increase in soil pollution poses a serious environmental threat worldwide. Anthropogenic activities, mostly industrial and agricultural, accelerate soil pollution [1]. Contaminated soils pose a major environmental problems, causing threat to groundwater reservoirs and food production [1,2]. Environmental pollution with heavy metals, caused mostly by industrial activities, is a worldwide problem and the progress of research on plant-based clean-up of contaminated soils such as phytoremediation is therefore of significant interest [3]. This inexpensive, plant-based and highly socially acceptable method takes advantage of the natural ability of plants to concentrate elements and compounds from the environment as well as to metabolize a vast range of molecules in their tissues [3,5]. Traditional technologies used for removal of soil contaminants can be successful in some situations, but these methods are expensive. Biological technologies like phytoremediation are more cost-effective, offering the use of plants natural properties to sequester, extract and purify contaminants [2,4].

Heavy metals as well as various organic pollutants are the major targets for pro-cess of phytoremediation. High levels of heavy metals can be hazardous to plants, animals and also to human health [3,5]. The main sources of anthropogenic soil contamination with metals include smelting, electroplating, energy and fuel production, the use of synthetic fertilizers in agriculture and industrial manufacturing [6]. Heavy metal contamination of the soils has increased significantly in last decades and poses major environmental problems worldwide [4,6].

Possibility to exploit plants for environmental remediation is restricted by our limited understanding of plant metabolic pathways and stress tolerance mechanisms [4,5]. At the moment little is known about the vast range of enzymes involved in the metabolism and transformation of most compounds that can be found in herbicides, pesticides, residues of explosive materials and industrial wastes [7]. Often the products of plant conversion remain unidentified. The natural ability of certain plant species to tolerate high concentrations of toxic compounds, detoxify and store heavy metals in their cells has a great importance for the development of phytoremediation technologies and phytomining applications [5,7,8].

Plant tissue culture is an useful laboratory tool for phytoremediation research. Most frequently applied form of tissue are cell suspensions and hairy roots [19,20]. Created *in vitro* cultures can be propagated almost indefinitely and are available on demand unlike the whole plants grown in soil, that have a limited lifespan and have to be grown separately for each experiment [21]. Therefore, the time necessary to carry out experiments can be significantly reduced using specified plant tissue cultures rather than whole plants. Additionally plant tissue cultures also offer many technical advantages compared with plants grown in soil [22]. Plant cultures are grown in sterile conditions and free from any microorganisms and can be used to distinguish between the responses of plant cells grown with and without microorganisms normally present in the rhizosphere or plant tissues [23]. In past years metabolism of many chemicals was considered to be beyond the enzymatic capacity of

plants and any level of biotransformation of these compounds in soil was attributed only to the actions of microorganisms linked with the plant roots. Plant cell cultures under sterile conditions has disproved this misconception and demonstrated that plant cells have the ability to metabolize a vast range of xenobiotics and other chemicals [14, 15,18].

Applications of micropropagation in phytoremediation studies

Phytoremediation is the utilization of plants for stabilizing or removal of pollutants from contaminated soil, water sediments and air [1].

The natural ability of plants to stabilize or remove pollutants from the environment and then to convert them into nontoxic or readily harvestable forms is used in every phytoremediation process [5, 9]. A vast range of hazardous contaminants (both organic and inorganic) can be taken up, conjugated and rendered harmless by plants. Substantial efforts are being made by academic, commercial and industrial groups to develop new practical technologies for use of phytoremediation process to treat contaminated soils, water and sediments [10]. Phytoremediation technologies allow avoiding the need for soil excavation and transport which make the process relatively cheap and cause less disruption of environment than chemical or physical remediation [9,10]. Plants can not only extract pollutants but also stabilize contaminated soil and provide optimal conditions for microbial activity in the rhizosphere [11,12]. However, one of disadvantages is that using plants for environmental clean-up usually takes more time than other remediation techniques and is most successful in areas where pollutants are present in higher layers within the reach of plant roots [13].

Micropropagation allows the production of large number of plants from the small pieces of a stock plant in relatively short period of time (figure 1). Depending on used plant species, the tissue explant may be taken from leaf, lateral bud, shoot tip, stem or root tissue [24]. Usually the original parent plant is not destroyed in the process, which is a factor of importance to the owner of a rare or unusual plant [25].

Possible applications of plant cell cultures in phytoremediation

The widespread application of micropropagation is the mass production of decorative and agricultural plants [26]. Usually conventional propagation is a time consuming process during which disease and pests can significantly limit production [26,27]. Micropropagation has the potential to produce hundreds, thousands or even billions of plants each year and offers several advantages that are not possible with conventional propagation techniques. Once established, *in vitro* cultures are a continuous source of cells and

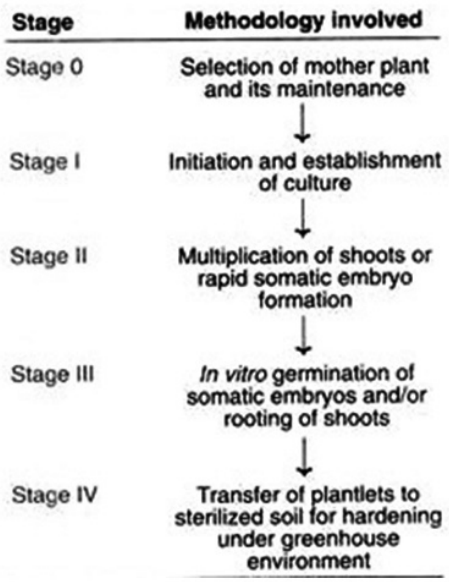

Fig. 1. Main stages of micropropagation [15]

tissues which can be used in plant production under greenhouse conditions without seasonal interruption [27,28].

Application of plant *in vitro* cultures provide the opportunity to control all conditions more easily than with plants grown on soil – particularly medium composition, phytohormone levels, nutritional parameters and provide the ability to manipulate the cells using different medium additives [26]. The ability to nourish *in vitro* cultures relatively high amounts of pollutants that would be unavailable from the soil allows the recovery of transformed metabolites and intermediates in quantities proper for mass spectrometry or nuclear magnetic resonance (NMR) analysis which provides a significant advantage research [29]. Because of the reduced amount of chlorophyll in plant cell culture in comparison to whole plants isolation of products from plant tissue cultures is easier and require fewer purification steps [29,30].

The use of *in vitro* plant cell or tissue cultures allows experiments to be carried out using material from the same parent plant, which allows to avoid the effects of variability between individual samples but somaclonal variation in plant tissues may introduce an independent source of variability. The relative homogeneity obtained in cultured cells and tissues can significantly improve the reproducibility of results in comparison to plants grown from seeds in soil [31,32].

Recent advances in understanding complex interactions between contaminants and plant could not have been achieved without *in vitro* plant cell culture techniques [30]. Application of such methods in phytoremediation studies is currently gaining more interest. Callus, hairy roots, cell suspensions and shoot multiplication cultures are currently used as models for better understanding of the uptake, localization, toxicity, and metabolism of pollutants under micro-

Tab. 1. Examples of the use of plants in vitro cultures in studies of organic contaminants

Pollutant	Plants species	Tissue culture	Reference
Polychlorinated biphenyls (PCBs)	*Solanum aviculare, Solanum nigrum*	Callus, hairy root	[33]
Phenol	Brassica *napus*	Hairy root	[34]
2,4-Dichlorophenol	*Nicotiana tabacum*	Cell suspension	[35]
3,4-Dichloroaniline	*Arabidopsis thaliana*	Root, cell suspension	[36]
TNT (2,4,6-trinitrotoluene)	*Nicotiana tabacum*	Cell suspension	[37]
RDX (hexahydro-1,3,5-trinitro1,3,5-triazine)	*Catharanthus roseus*	Hairy root	[38]
Metamitron	*Nicotiana tabacum*	Cell suspension	[39]

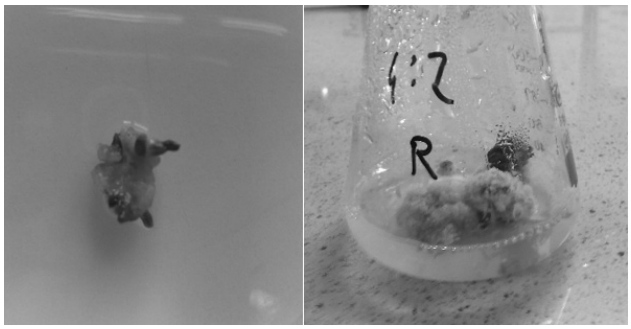

Fig. 2. In vitro cultures of Robinia pseudoacacia (photo M. Jaskulak)

bes-free conditions [33]. Moreover, such cultures can be propagated indefinitely and available through all seasons [34]. In contrast, whole plants grown in soil have a limited lifespan and needs to be replaced and reestablished after each study. Using plant tissue cultures can significantly reduce the time required to carry out experiments [35]. It is worth mentioning that plant tissue culture is also a necessary step in most on the genetic transformations. Therefore, the development of transgenic plants that can tolerate or even degrade for example xenobiotic compounds would not be possible without *in vitro* cultures [36]. Besides strictly technical convenience, plants in vitro cultures can be used to distinguish the different metabolic capabilities of plants cells from those of microorganisms. For years the metabolism of xenobiotic compounds was considered way beyond the enzymatic capacity of plants and the use of plant cell cultures were able to establish that plants can metabolize a vast range of xenobiotics [37,38].

Hairy roots cultures

Hairy roots cultures provide additional features and benefits relative to plant tis-sues such as callus and suspended cells that can be used in phytoremediation [33]. Due to the fact that plant roots are in direct contact with pollutants in contaminated water or soil, their responses to toxic chemicals are more immediate [33,34]. Re-search performed using separately cultured organs such as hairy roots allow the ac-cumulation or biotransformation capabilities of roots to be identified without interference from translocation effects. This allows to determine, for example, whether translocation is necessary for accumulation or metabolism of specific pollutants [35]. Hairy roots offer many advantages of genotypic and phenotypic stability compared with different plant cells or tissue cultures, thus providing a more reproducible and reliable experimental system over time. They also have simpler culture requirements then other plant cultures [36,37].

Toxicity and tolerance studies

The resistance or susceptibility of plants to various pollutants, both organic and inorganic, depends mostly on how well intake and metabolism of the pollutant is executed and controlled [38]. All these processes can function differently in plant cell/tissue cultures in comparison to whole plants and can exhibit different toxicity and stress tolerance properties. However, several research studies demonstrate that plant tissue cultures can be useful and reliable experimental models for toxicity assessments and tolerance studies [38, 39].

For example different soybean cultivars that were either herbicide-resistant or highly herbicide-sensitive retain their natural properties in performed suspension culture and differences in pesticide and herbicide toxicity observed between plant cultivars were also maintained *in vitro*. In another example hairy roots cultures of Cd and Ni hyperaccumulating plants retained their enhanced ability to tolerate high concentrations of metals in relation to non-hyperaccumulator species. Other similar results occurred with callus initiated from a Cd-tolerant species of different fern species: tissues grown *in vitro* also exhibited Cd resistance [40,41,42].

Observed correlation between the tolerance properties of *in vitro* cell cultures and regenerated plants allows plant cultures to be used in the selection of metal or xenobiotic resistant clones [16,17]. This approach was used in breeding herbicide-resistant plant lines and plants with superior tolerance to toxins or accumulation of heavy metals [43]. In other studies aluminum-resistant plants were developed from tissue culture divided into groups differing in concentration of Al, illustrating that somaclonal variation in plant cultures itself was able to induce metal resistance. Increasingly plant cell/tissue cultures are being applied to investigate the antioxidative stress responses of plants to a vast range of contaminants including toxic heavy metals. In studies comparing the antioxidative responses of callus cultures and whole plants grown in soil to high Cd concentrations, the levels of main antioxidative enzymes were found to be higher in callus cultures than in normal plants and were correlated with significantly greater cell survival rate [44,

45]. In experiments with plants hairy root cultures antioxidative defences have been implicated as a factor with strong contribution to the metal-hyperaccumulator phenotypes of *Alyssum bertolonii* and *Thlaspi caerulescens* [46].

The ability to manipulate the parameters of growth medium for easy application of inhibitors and chemical effectors is a strong advantage associated with plant cell/tissue cultures in many toxicity studies compared with plants grown on soil [46,47]. A vast range of metabolic inhibitors helped in explaining the mechanisms of metal tolerance and toxicity in plants. For example buthionine sulfoximine (BSO) was used in research to determine impact of glutathione or phytochelatins on metal tolerance in hairy roots cultures and suspended cells . Diethylstilbestrol (DES) has also been applied to test the effects of membrane depolarization on heavy metal up-take in hyperaccumulator plants [47,48].

Creating pathogen-free plants and meristems cultures

Plant *in vitro* tissue culture is often used in industry for the obtaining and mass production of specific pathogen-free plants through meristem culture process [49]. Meristem culture was first created and pioneered by Morel (1960) and involves the removal from parent plant and placing the meristem culture on a growth medium. The meristem is a set of actively dividing cells, approximately 0.1 mm in diameter [50]. Most endogenous contaminants cannot invade the meristem which often results in the formation of a disease-free plant. This process can be easily combined with micropropagation technique in result a large number of disease-free plants may be produced from meristematic cultures [49,50].

Techniques of meristem culture has been used successfully in the removal of many viruses posing a threat to the plants (potato, sugarcane, strawberry) and is now often used routinely for the suppression of many viral diseases. Techniques of meristem culture have been used successfully to obtain virus-free plants in a vast range of plant species and bacteria-free plants of species known to have certain leaf spot diseases [49,50,51].

Selection of plants resistant to environmental stress

Today one the most heavily researched area of plant *in vitro* culture is the possibility of isolating, selecting insect, disease or stress resistant plants [52]. Search for superior individuals, resistant to environmental threats can be vastly accelerated using *in vitro* techniques. Such systems provide the opportunity to exploit the existing, natural variability in plant species or in some cases variability can be induced by different chemical or physical agents known to cause mutations [53].

Natural variability in nature is a major advantage in finding resistant plants. For example, even in frost-tender species, certain cells or groups of cells may be frost hardy [54]. However, because most of the organism is killed by frost, the tolerant cells eventually die because they are unable to support themselves without the remainder of the organized plant [54,55]. If these groups of cells are then subjected to a selection agent such as freezing, tolerant ones can survive while all susceptible will be killed. This concept can be applied to many types of stress as well as resistance to fungal and bacterial pathogens and various types of phytotoxic chemical agents.

The goal of selecting such resistant cell lines would be to reorganize in whole plants from them which would retain the selected resistance [55,56]. Current re-search in this area extends across many interests including attempts to select salt tolerant lines of tomato, freeze resistant tobacco plants, herbicide resistant agronomic crops and various species of plants with enhanced pathogen resistance [57].

Limitations of using *in vitro* cultures

Despite shown advantages offered by plant cell or tissue cultures in phytoremediation research *in vitro* culture is often is too expensive for direct application on a large-scale phytoremediation operations. *In vitro* cultures require sterile culture conditions and contamination of plant cultures with fungi or bacteria can lead to significant losses in plant culture [58,59]. *In vitro* plant cultures are typically heterotrophic and require supplementation of sugars in the growth medium so the potential for microbial contamination of the cultures under non-aseptic conditions is extreme [60].

Commonly used remediation applications where inexpensive agricultural waste products such as corn stover, wood chips and organic fertilizers are effective for removal of contaminants from the soil while large-scale plant tissue culture is far from commercially profitable [59,60]. The key role of plant cell or tissue culture in phytoremediation studies is as a model system for understanding the metabolic and tolerance mechanisms that exist in plants and also to create pathogen-free species and selecting plant resistant to studied contamination, stress or other environmental factor [60,61].

Conclusion

1. Environmental pollution is a serious threat to not only to ecosystems but also mostly through food production to human health. Widely spread pollutants include fertilizers, pesticides, heavy metals, trichloroethylene, halogenated phenolic, and other waste products. Agricultural and industrial contaminants are becoming a worldwide problem which is the reason that there is increasing interest in plants that accumulate, detoxify, or degrade these substances.

2. Plant tissue culture is defined as the culture of plant cells, tissue or whole organs under sterile conditions. Plant *in vitro* cultures play an important role in phytoremediation research mostly by extending our understanding of plant metabolism [49]. Cell and tissue cultures offer more than just an ease and speed in comparison with whole plant systems [62]. They eliminate negative effects of microorganisms and translocation barriers and in results closer approximation of the ability of plant tis-sues for detoxification of contaminants can be obtained. If a studied pollutant is metabolized by plant cell *in vitro* culture it is a clear indication that the specific plant has the genetic capacity to biotransform that compound [62,63]. Although plant tissue cultures have a significant value in studies of metabolism, toxicity and stress tolerance, they are not a replacement for soil-grown plants – they are just a more precise model that can be exploited to obtain useful information to guide subsequent whole-plant trials [64]. Plant tissue culture studies offer prospects to future improvements in crop productivity and quality. And while most commonly used technology in plant *in vitro* cultures is micropropagation, other types of tissue culture research are less publicized for example plant cell cultures can be used to select pathogen or stress-resistant plant clones [65]. Recently, interest is growing in the possible use of plants for phytoremediation of soils that are contaminated with heavy metals or other contaminants like for example petroleum. The main requirements for an applicable large-scale propagation system for plants are cost effectiveness, reproducibility and simplicity.
3. Many dimensions of tissue culture research have been less well publicized. The potential for the selection of pathogen free, stress-tolerant and pathogen-resistant clones of plants and the novel genetic combinations to be achieved through somatic hybridization are all lines of research which can have a profound impact on environmental science.

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