LECTURES

Plant biotechnology and *in vitro* culture – the fulfilled hopes?

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The *in vitro* culture is an intrinsic component of a single area of plant biotechnology, the one dealing with cloning. The *in vitro* industry maintains a strong position in the economy and is developing well. Cloning is also a stage required for recovery of genetically modified plants but in this regard it is highly imperfect. There are also fields of biotechnology in which prospects of utilizing solutions offered by *in vitro* cultures once appeared very promising but have so far not been fulfilled, and it seems that things will remain that way. What are the reasons of this failure? Did we mis-estimate the potential of *in vitro* cultures? Is it rather the result of a more general trend, or do the causes lay entirely somewhere else?

The lecture will attempt to address these questions by analyzing specific circumstances and experimental data. It will also try to highlight key problems within the field of *in vitro* cultures, that, if solved, could likely benefit particular subdisciplines of biotechnology.

What's new in the control of bacterial contamination in plant tissue cultures?

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There are three main issues in the control of bacterial contaminations in plant tissue cultures: 1. preventing the introduction of bacteria with the initial plant material, 2. preventing the introduction of bacteria from the environment during subculturing, 3. reducing bacterial contamination in the cultures at the stage of shoot multiplication and rooting.

Problems with bacterial contamination in *in vitro* cultures will probably never be fully resolved due to the unlimited quantity and diversity of bacteria in the environment. At present, the lack of satisfactory characterization of bacteria often makes their identification and control impossible. Furthermore, bactericidal compounds are often damaging to plant tissue cultures.

The most effective way of preventing bacterial contamination *in vitro* is elimination of bacteria from the initial plant explants that are introduced into the cultures. This is not easy, especially with endogenous bacteria, which are perfectly adapted to their hosts. The most promising methods involve the use of precultures of donor plants under a strict sanitary regime and efficient sterilization of the initial explants, reduction of the size of the initial explants just to meristematic tips, and early detection of bacteria in the isolated tissue.

In plant tissue cultures propagated for a long time or in cultures stored over long periods, it is possible for bacteria from the laboratory and human environment to accumulate. Such microorganisms can occupy their own niche in the culture vessels or on/in explants. Occasionally, they can also be sources of human pathogens. In such cases, the most effective solution is careful and frequent inspection of the cultures and the maintenance of a reserve of clean cultures under strictly protected conditions.

Sometimes, bacteria emerge in great quantities at the stage of intensive shoot multiplication or rooting, affecting a large number of microplants. In this case, the obligatory procedure is to eliminate cultures containing the deleterious bacteria (including pathogenic species) and to add bactericidal compounds, that are not detrimental to the explants but that can diminish the bacterial population, to the media of the other cultures.

In the lecture we will present illustrations from the most recent reports on this aspect of plant culture.

FlaxAid, a new wound dressing based on transgenic flax products

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Recent reports have indicated that oxidative stress could be an important mechanism aggravating the chronic wound progression. It has been suggested that reactive oxygen species are responsible for chronic wound pathogenesis and anti-healing processes because of the reduction in proliferating capacity of wound cells exposed to oxidative stress. Oxidative stress causes damage to cellular macromolecules, down-regulates key proteins involved in DNA replication, de-regulates the cell cycle, increases the cellular resistance to oxidative stress and promotes apoptosis in wound fibroblasts. Multidirectional analysis has also revealed that chronic wound fibroblasts have a decreased ability to withstand oxidative stress. In agreement with this is the report providing evidence that antioxidative compounds, such as catechin, promote the healing of chronic gastric ulcers.

In the present study we propose a new material and method of treatment of trophy lesions based on products from transgenic flax plants. The transgenic plants overproduced antioxidative compounds of different types. Characteristic features of those plants were an increase in phenolic contents in fibres and unsaturated fatty acids in seeds, and a strong increase in lignan in seedcake. It was expected that a coordinated use of fibres, oil emulsion and seedcake extract from transgenic plants will promote the healing of chronic skin ulceration. Therefore the primary goal of this study was to investigate the clinical improvement in chronic trophy lesions by investigating changes in wound exudation and the proportion between fibrin level and granulation level. Another goal of this study was to test the effect of FlaxAid wound dressing on wound size and the pain usually accompanying chronic ulceration.

FlaxAid wound dressing is a three-component product based on fibres, oil emulsion and seedcake extract from genetically engineered flax plants. All these materials are derived from two types of transgenic flax plants which were obtained by plant transformation using three genes controlling the synthesis of antioxidative compounds from phenylpropanoid pathway and genes responsible for hydrophobic polymer synthesis. Simultaneous transformation of flax explants with three genes coding for chalcone synthase (*CHS*), chalcone isomerase (*CHI*) and dihydroflavonol reductase (*DFR*) resulted in accumulation of phenolic acids in fibres, polyunsaturated fatty acids in oil and lignans in seedcake. Simultaneous expression of three bacterial genes in flax vascular bundles resulted in synthesis of cellulose-polyhydroxybutyrate composite fibres. Those three products (fibres, oil, and seedcake) of transgenic flax that contained a broad spectrum of antioxidative compounds were tested for cytotoxicity and were sequentially used for chronic wound healing. None of these products showed negative effect on growth and morphology of Balb/3T3 cells in the cytotoxic assay.

In this report we present the effects of linen dressing treatment alone and in combination with oil emulsion and seedcake extract on chronic wound healing in patients. A twelve week application of FlaxAid wound dressing gave a faster healing and, specifically, a reduction in wound exudates and wound size. In several cases wound healing was completed during the period of investigation. Interestingly and importantly, FlaxAid wound dressing diminished the pain accompanying chronic venous ulceration as reported by patients. The molecular mechanism of this effect was based on the action of cannabinoid (CBD), respective CB2 receptor activation and suppression of pro-inflammatory cytokine.

The beneficial effect of FlaxAid wound dressing on wound healing is reported here for the first time.

GMO – A.D. 2009

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Modern biotechnology plays a critical role in the knowledge based bio-economy. For further development of bio-economy we need science and transfer of innovation from academia to industry as well as supportive legislation and public acceptance. Majority of members of the Polish society are against genetic engineering and majority of experts are "pro" innovative technology. The Government is in favor of "GMO free zones" in contrast to the EU Commission's support for GMO.

Quo vadis Polish biotechnology?

Biology of cell wall-plasma membrane-cytoskeleton continuum in plants

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Cell ***w***all-plasma ***m***embrane-***c***ytoskeleton (WMC) continuum in plants is one of the major integrators of the structure and function of plant cells. Although spread throughout the cell, it also smoothly amalgamates into higher level structures, such as the apoplast, and thus constitutes an important determinant of plant's organismal features. Owing to the cell walls, it constitutes a major barrier between protoplasts and their surroundings, but it also is a dynamic entity participating in sensing of and responses to internal and external signals.

Recent years have brought many interesting observations demonstrating the importance of WMC continuum in plants. Here, several major aspects will be presented and discussed. First, the continuum is deeply involved in signalling processes, among them those that affect the fate of individual cells as well as those that influence the growth and development of the whole plant. Moreover, more and more signalling molecules are identified that originate from the walls. Second, the cell walls should no longer be considered as an invariant structure around the protoplast. They undergo recycling and remodelling, enabling a precise regulation of mechanical and chemical properties of the various wall domains. These processes are under a tight control by the cytoskeleton, and involve also intensive vesicle trafficking. Third, the continuum seems to constitute the major sensor and transducer of mechanical stimuli. This enables the control of the cell shape and, to some extent, function which finally results in modulations of cellular fate. All these aspects will be illustrated with data coming from the work in our lab.

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