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# ELECTROPHYTOCHEMISTRY - AN EMERGING FIELD OF ACTIVITY

*KSA GNANASEKARAN*
Central Electrochemical Research Institute, Karaikudi 623006

ABSTRACT

Electrophytochemistry is an interdisciplinary area that deals with the electrical and electrochemical aspects of plant science. Some of the basic techniques involved are (i) the measurement of cell potential (transmembrane) using microelectrodes (ii) the monitoring of plant-body potential embedding noble metal electrode in conjunction with a reference electrode set in the soil and (iii) fusion of cells by electrical induction. Membrane studies are useful in knowing the transport of metabolytes. The variation of plant body potential with time (electrophytogram) can be related to the gross physiological activity.

Electroculture remains fascinating though benefits on a large scale cannot be confidently predicted. Ionoculture seems promising in improving plant health.

Electrophytochemistry will play a vital role in the development of biotechnology such as biocatalytic hydrogen production and use of chlorophyll membrane in constructing photoelectric cells. The electrically induced fusion of cells and entrapment of membrane impermeable substances and genes in them provide a new tool for the production of a wide range of cells with manipulated functions, which are used for the solution of problems in cell biology, medicine and technology.

**Key words: Electrophytochemistry, electrophytogram, electroculture**

## INTRODUCTION

Electrophytochemistry deals with the electrical and electrochemical aspects of plant growth. It embraces broadly soil science, plant science and atmospheric science. As a plant is a product of its environment, the soil-plant-atmosphere continuum is to be studied on the whole for a better understanding.

### The electrical and electrochemical potential in plants [1]

*a) Measurement of electropotentials across cell membranes*

Electrical membrane potentials are measured by the aid of glass microelectrodes whose outer tip diameter is of the order of one micron for small cells, but $2-20\,\mu m$ for large algal cells. The glass electrodes used for insertion are usually filled with 3M KCl solution. The electrodes are introduced into the cell by micromanipulators so that the electropotential difference between the cell interior and the external solution can be measured. Generally the electrode tip is inserted into the cell vacuole giving the PD between the vacuole and outside, $E_{vo}$. Thus this PD is across the tonoplast and the plasmalemma as well as the cell wall. A reference electrode is immersed in the external solution. The RE and the insertion electrode are connected to a sensitive electrometer having a high input impedance ($\geq 10^{10}\,\Omega$).

Fig. 1 shows the experiemental set up for transmembrane electropotential measurements.

$$E_{cell} = E_{v\rightarrow O} = E_{c\rightarrow O} + E_{v\rightarrow C}$$

where,

$E_{v\rightarrow O}$ = the potential between vacuole and outer solution

$E_{v\rightarrow O}$ = the potential between cytoplasm and outer solution

and $E_{v\rightarrow O}$ = the potential between vacuole and cytoplasm

*b) Action potential in plants*

External electrical, mechanical or chemical stimuli can transiently depolarise the membrane potential in analogy with animal systems and are known as action potentials. The basic molecular processes during action potentials in animals and plants do not seem to be different in principle. The resting membrane potential usually is much more negative in plant than in animal cells. On the other hand, responses and conduction of action potentials are much more sluggish in plant cells.

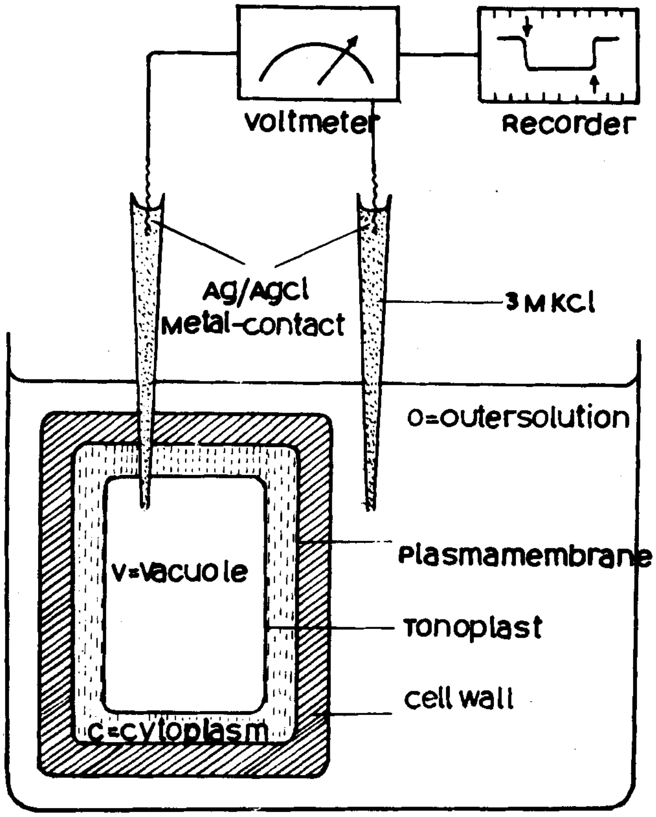


Fig. 1 : The experimental set up for transmembrane electropotential measurement.

Nevertheless, there are only differences of degree between plant and animal systems; the more primitive animal systems display responses on the same order as the fastest plant reaction. In animal nerves, the ionic basis of action potentials is given by fluxes, permeabilities and electrochemical gradients of $K^+$ and $Na^+$ ions, whereas in plant cells $K^+$ and $Cl^-$ ions are involved. $Ca^{2+}$ ions are apparently needed for the initiation of action potential. Also in some animals, $Cl^-$ ion fluxes are involved, as in plants, for the action potential.

*c) An electrochemical instrumentation system for agriculture and plant sciences [2]*

The basic technique in this instrumentation system is the placement of an intrusive noble metal electrode in the stem or petiole of higher plants. A reference electrode is placed in the soil. Potentials of botanical origin are measured between the electrodes. The circuit path consists of the reference electrode interface, soil, soil-root interface, roots, stem and tissue monitoring probe interface. There is evidence that a substantial portion of the potential variations encountered can be attributed to variations in the vicinity of the tissue monitoring probe interface.

The form of this electropotential variations can be divided into different catagories, viz. (i) healing potentials (ii) transition potentials (iii) diurnal high magnitude oscillations (iv) high frequency low magnitude oscillations (v) water status related potentials and (vi) negative going bursts of relatively variable duration. The long term display of the DC potential against time, called an Electrophytogram (EPG) can be related to gross physiological acitivity.

There are still problems with this technique of getting EPG that must be recognised. The specific electrochemical basis of the potential is poorly defined. Furthermore the potential is not spatially defined. However, if the electropotentials are related to cellular activity, and the data certainly points that way, as well as water status, the applications are myriad. Growth, water status and genetic selection are but a few.

**Electroculture [3]**

Electrostatic fields ranging from 100 V $m^{-1}$ to 800 kV $m^{-1}$ have been applied to plants under laboratory conditions and in field trials since 1880. Some beneficial effects have been recorded (e.g. increase in yield from both cereal and vegetable crops); but the results have been nonuniform. The electrical conditions leading to definite benefits on a large scale cannot be confidently predicted from early studies.

The effects of microwave radiation (mostly 2450 MHz) upon seeds, plants and soils have been studied. These effects depend upon the power density of the radiation and the electrical properties of the targets. Factors such as size of the seeds and plants, shape and moisture content are important, as are the properties of the soil irradiated (notably water content). Microwave radiation can kill weeds and seeds that are buried several cm deep in the soil. Small currents ($10^{-7}$ to $10^{-2}$ A) passed through plants and leaves produce a variety of responses. This can affect the electrical resistivity of the plant tissue, growth rates and movement of metabolities. Heavier current (i.e. 50 mA and above) cause the destruction of the plant tissue by rapid heating of cellular water leading to cell membrane rupture.

**Ionoculture [4]**

In the electroculture and related experiments air-ions play a large role. Other factors such as ozone formation, nitrogen oxide formation, humidity and air pollutants affect the results Hence careful monitoring is essential when growing plants in ion-chambers to study the effect of air-ions on them. The normal concentration of small air-ions in normal air is about $10^3$ to $5 \times 10^3$ ions/ml. Because of rapid recombination mechanism, the upper practical limit inducible by external means is about $10^6$ ions/ml. It may be noted that air has about $27 \times 10^{19}$ molecules/ml under normal conditions.

Air-ions have marked influence on the physiological processes of plants and mammals. The dominant positive small ions in air are present as the hydrated form of $H^+$, or as the negative ions, $O_2^-$, $OH^-$, $CO_3^-$ and $CO_2^-$. Air-ions are said to guide or enhance the synthesis of cytochrome C and other iron-containing enzymes.

## Biotechnological application [5]

*a) Hydrogen production*

It is possible to produce hydrogen by a bio-catalytic process, in which water is decomposed by photolysis using chlorophyll and an enzyme, hydrogenase. Such a scheme is presented in Fig. 2 below:

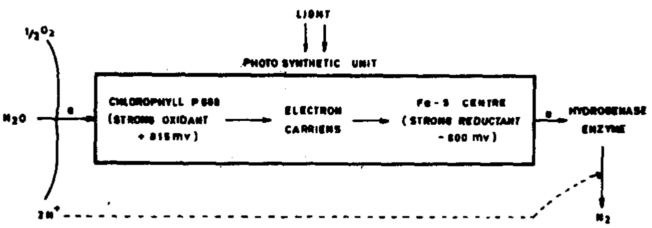


Fig.2. Production of hydrogen by a bio-catalytic process.

Certain algae produce hydrogen gas under specific conditions; they contain the enzyme hydrogenase. With the enzyme hydrogenase any plant type system could have the ability to produce hydrogen gas. This has been demonstrated in the laboratory using components extracted from leaves and bacteria.

*b) Artificial chlorophyll membranes*

The chlorophyll containing membranes of all photosynthetic organisms are able to separate positive and negative charges on either side of the membrane under the influence of light. The basic photogalvanic system is a key to photosynthesis which we might be able to use directly for the production of electricity or the storage of energy. Artificial chlorophyll containing membrane bilayers and vesicles have been used and shown to produce current and charge separtion. The possibility of using such membranes for direct photochemical systems has scope.

## Cells with manipulated functions [6]

Exposure to electrical fields can reversibily increase the electrical conductivity and permeability of a cell membrane, which regulates and directs the exchange of materials and information between the cell and its environment. If cell membranes (or artificial lipid membranes) are exposed to a field of high intensity and short duration (ns to $\mu$s) local electrical breakdown occurs in them. This is associated with a large permeability change in the membrane. Substances or particles (upto the size of genes) which cannot normally penetrate through the membrane, are then able to transverse the cell membrane into the cell. The original properties of the membranes are restored within microseconds to minutes depending on the experimental conditions and the membrane properties.

Electrical breakdown in the zone of contact between the membrane of cells (or lipid vesicles), which have been made to adhere to each other by the action of weak inhomogeneous alternating electric fields, leads to fusion of these cells with formation of a single cell having new functional characteristics. The electrical fusion method is very mild, and the yield of fused cells is very high.

The electrically induced fusion and entrapment of membrane-impermeable substances and genes in cells provide a new tool for the production of a wide range of cells with manipulated functions, which could be used (or being used) for the solution of a number of problems in cell biology, medicine and technology.

Fusing plants protoplasts of different origins to produce crop plants with new properties, such as improved yield or better salt tolerance, is quite conceivable. Salt-tolerant mutants of the soya bean are well known. Several laboratories are thus attempting to fuse protoplasts of this mutant, with protoplasts of other crop plants in order to produce a viable cell hybrid which can be used to breed plants having the combined properties of both.

The transfer of the gene responsible for nitrogen fixation from bacteria to plant protoplasts with the aid of the electrical fusion method is also coming into the realms of possibility. The fusion of protoplasts from yeast cells with plant cells is another fascinating area which could find technological application. Yeast cells are able to convert sugar into alcohol. By immobilizing yeast cells in cross linked polymer matrices, it is possible to produce alcohol in a continuous process. Plant protoplasts on the other hand are able to convert solar energy into starch. A hybrid cell with the properties of both cells should thus be capable of converting solar energy directly into alcohol. If it were possible to operate such a cell system on a large scale, the implications for the solution of our current energy problems would be enormous.

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