2 Electrochemical Methods and Measuring Transmembrane Ion Gradients

ANTHONY J. MILLER, DARREN M. WELLS

2.1 Methods for electrical recordings from plants

2.1.1 Making contact

The measurement of a voltage requires a complete electrical circuit or ring that includes the measuring device, a voltmeter or electrometer. The electrical contact to the biological material is provided by an electrode. This interface between the biological specimen and electrometer is very important, as ideally it should provide a low electrical resistance pathway that does not interfere with the cells or tissues being measured. The word *microelectrode* is commonly used to describe a glass micropipette which is pulled into a fine tip at one end and filled with an aqueous salt solution. The junction between the salt solution inside the microelectrode and the input to the electrometer amplifier is provided by a half-cell. There are different types of half-cell, but usually the metal contact is AgCl-coated Ag wire and the salt solution is 0.1 M KCl (e.g. World Precision Instruments, Sarasota, Fla., USA: http://www.wpiinc.com/). The micropipette provides a salt bridge between the inside of a living cell and the metal contact in the half-cell. The simplest microelectrodes measure voltage and when inserted into cells measure the membrane potential, in mV, between the inside and outside of the cell. The metal contact can be made directly to the cell or tissue surface, but this type of electrode can be subject to various types of interference as the surface can be coated by plant material that will influence the stability and size of the electrical potential reported. This problem is much less likely to occur when the tip is constructed from glass that has been heated and pulled into a small fine tipped microelectrode. A small tip also provides less intrusion and interference for the biological tissue or cells being examined.

An *ion-selective* microelectrode contains an ion-selective membrane in the tip of the glass micropipette and is responsive both to the membrane potential and the activity (not concentration) of the ion sensed by the selective membrane. To make intracellular measurements, it is necessary to also simultaneously measure the membrane potential either by insertion of a

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Crop Performance and Improvement Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

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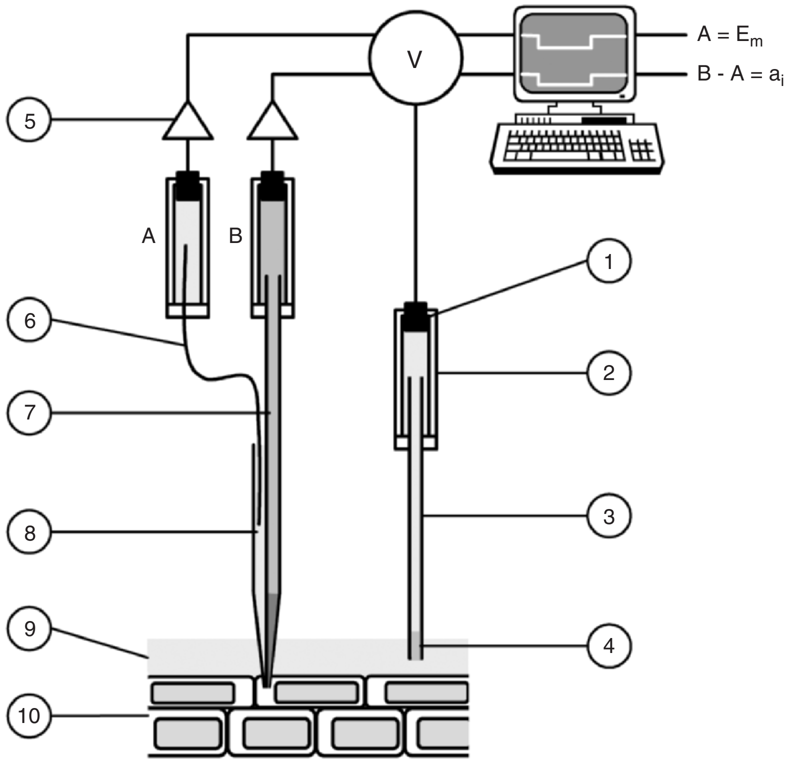

Fig. 2.1. Diagrammatic representation of a double-barreled ion-selective microelectrode. *1* Ag/AgCl chloride coated pellet, *2* half-cell, *3* salt bridge, *4* porous glass frit or agar plug, *5* head-stage signal amplifier, *6* Ag/AgCl coated Ag wire, *7* ion-selective barrel with sensor plug in the tip, *8* cell membrane potential barrel filled with 0.1 M KCl, *9* nutrient solution bathing plant, *10* plant tissue with microelectrode tip in a cell (cytoplasm)

second electrode or, for small cells, by combining the ion-selective and voltage-measuring electrodes into a *double-barreled microelectrode* (see Fig. 2.1). In order to be able to measure several different ions, it may be necessary to combine together several different electrodes to make multi-barreled electrodes (e.g. Walker et al. 1995).

Solid metal electrodes have been used to directly report from plant material and for some types of specialist uses such as measurements of electrical current in oxygen electrodes. Metal electrodes are usually made from Ag or platinum and these solid state electrodes have been used to make ion-selective microelectrodes (see section 2.2). Metal electrodes have also been used for direct recording from the surface of plants to measure extracellular transient electrical signals such as those elicited by external signals, e.g. wounding. The interface between the plant material and a metal recording electrode may also