

4 Electrochemical Sensor Applications to the Study of Molecular Physiology and Analyte Flux in Plants

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4.1 Introduction

Electrophysiological studies on plants have always been challenging. Cell walls and turgor pressure have made it difficult to apply many standard approaches used on animal cells. For these reasons, plant physiologists have long sought and developed novel methods for examining cellular physiology via non-invasive approaches. This review covers one such example, an electrochemical method that evolved out of a series of capacitively coupled techniques using a modulation approach. The first, for biological applications, was published by Blüh and Scott (1950) using an aerial probe. Although sparse on results, the authors claim preliminary data mapping surface potentials from various plant materials. Further development was reported in a series of papers by Grahm (1964) and Grahm and Hertz (1962, 1964) with data on the geoelectric and auxin-induced effects from coleoptiles using parallel plate capacitors. Recently, a more refined device, termed the bioKelvin probe, measured light-induced field changes originating from subsurface currents of a single corn coleoptile (Baikie et al. 1999). This device incorporated positional feedback and improved spatial resolution. Sanger et al. (1990) published a preliminary report using a similar approach.

The biggest breakthrough in the design of an instrument for monitoring external voltage fields, as related to the transmembrane passage of ions, came with the full development of the vibrating voltage probe by Jaffe and Nuccitelli (1974). Davies (1966) briefly described a similar device applied to muscle fibers. While subsequent studies by Jaffe, colleagues, and others are too numerous to mention, the vibrating probe was put to good use by plant physiologists as a method for monitoring ion transport (reviewed by Nuccitelli 1991).

Although a powerful tool, used to great effect on studies involving patterning and tip growth, the voltage probe has the limitation of not being chemically selective. An approach was needed where identification of the transported ion(s) that generated the current could be achieved. The first example was published by Gow et al. (1984), measuring H⁺ gradients over the

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surface of growing hyphae, with the landmark paper by Jaffe and Levy (1987) automating the process for Ca^{2+} measurement. The full description was published by Kühtreiber and Jaffe (1990), with diversification to K^+ , H^+ , and Ca^{2+} fluxes in maize roots and maize suspension cells by Kochian et al. (1992). Around the same time further plant applications were being explored by Newman and colleagues (Arif and Newman 1993). The use of ion-selective electrodes (ISEs) in this configuration is termed self-referencing by the authors (Smith et al. 1999). Over the approximately 20 years since its development there have been numerous successful applications and diversification to include other liquid membrane sensors, Cd^{2+} for example (Pineros et al. 1998) and Cl^- (Shabala and Newman 1998; Shabala et al. 2000). Cl^- detection is of particular interest to this review, and will be considered below as detection with the available Cl^- -selective solvents is problematic, as recognized by Doughty and Langton (2001), Messerli et al. (2004), and Garber et al. (2005). The use of these sensors highlights the problems and pitfalls that can befall the unwary investigator in applying any electrochemical sensor.

Following the potentiometric ISEs came the self-referencing amperometric design, first applied to oxygen detection (Land et al. 1999; Mancuso et al. 2000). These solid-state redox-based sensors have so far seen a broader application in the animal sciences, but the potential to measure nitric oxide flux (Kumar et al. 2001; Porterfield et al. 2001) or H_2O_2 efflux (Twig et al. 2001, 2005) offers a clear application in plants. Modification through enzyme incorporation has also been demonstrated (Jung et al. 2001).

Electrochemical sensors, when used correctly on biological systems, offer a huge experimental return, particularly in the plant sciences where more conventional electrophysiological approaches are difficult. The self-referencing approach enhances this return by providing flux data and powerful signal analysis, refining sensitivity and selectivity. This review aims to introduce the reader to both the strengths and the weaknesses of this technique, which, although now 2 decades old, may still be unfamiliar to many.

4.2 Electrochemical detection with microelectrodes

4.2.1 Properties of electrochemical sensors

Self-referencing with electrochemical sensors is a modulation technique where a single sensor is used to measure the activity/ concentration of an analyte at different points in space and time. As micron-sized electrochemical sensors are the basis for self-referencing, we will discuss some physical properties of these sensors before discussing their use in a self-referencing mode.

The two fundamental types of micro-electrochemical sensors used with self-referencing to date are the potentiometric, ion-selective electrodes (ISEs), and the amperometric microelectrodes. The ISEs are based on an ion-selective