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Transmembrane Potential Measurements on Plant Cells Using the Voltage-Sensitive Fluorescence Dye ANNINE-6

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Recently, effects of pulsed electric fields on plant cells have become an interesting research topic in plant electrophysiology.
Application of an external electric field leads to membrane charging up to a distinct transmembrane potential, where a strong membrane permeability increase prevents further charging of the plasmalemma. A common explanation for this increased permeability is the enhanced formation of pores in the lipid bilayer allowing ions and other molecules to pass the cell membrane.
Protoplasts prepared from the cell line *Nicotiana tabacum L. cv. Bright Yellow 2 (BY-2)* have been stained with ANNINE-6, a fast voltage-sensitive dye which exhibits fluorescence intensity changes based on electronic transitions due to the molecular Stark effect within a subnanosecond time range. For the excitation of the fluorescence dye, a 5 ns laser pulse with a wavelength of 468 nm delivered by a Nd:YAG-pumped dye-laser has been used. The voltage pulse driving the external electric field is provided by a microscope-based Blumlein-line generator.
The measured saturation of the fluorescence intensity is a clear sign for the limitation of the membrane charging by the formation of pores.
The field strength dependence of the protoplast's transmembrane potential V_M shows strong asymmetric saturation characteristics due to the high resting potential of the plants plasmalemma of approximately -150 mV.
The azimuthal dependence of the transmembrane potential, measured in angular intervals of 10° along the circumference of the cell, shows a nearly sinusoidal shape for an undisturbed membrane. Application of higher field strengths leads to a flattening at the pole regions due to the beginning formation of pores. Additionally, at the hyperpolarized cell pole a polarization reversal could be observed at an external field range around 1.0 kV/cm. This behaviour might be attributed to a fast charge transfer through the membrane at the hyperpolarized pole, e.g. by voltage gated ion channels.

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