# The Bioelectromagnetics Society
# 29th Annual Meeting
# Abstract Collection

## Kanazawa-shi Bunka Hall
### Kanazawa, Japan
### June 10 - 15, 2007

## **5-8** PULSED ELECTRIC FIELDS PROMOTE POTATO TUBER CELL WALL CROSS-LINKING

P. Thomas Vernier[1,2], Federico Gómez Galindo[3], Petr Dejmek[4], Antonio Vicente[3], Martin A. Gundersen[2]
[1]University of Southern California, Marina del Rey, CA, USA [2]University of Southern California, Los Angeles, CA, USA [3]Universidade do Minho, Braga, Portugal [4]Lund University, Lund, Sweden

**Objectives.** Pulsed electric fields affect plant tissue not only by permeabilizing cell membranes but also through pulse-induced modifications of the cell wall. By monitoring cell membrane staining with FM1-43, which fluoresces intensely in phospholipid bilayers [1], we tracked increases in cross-linking (and reduction of porosity) in potato tuber cell walls after exposure to 1 ms rectangular electric pulses ranging in amplitude from 3 kV/m to

50 kV/m. A decrease in the FM1-43 diffusion rate through the cell wall occurs within 30 seconds after pulse delivery. This response is mimicked by exogenous $H_2O_2$ and is blocked by sodium azide, an inhibitor of the peroxidase-catalyzed production of $H_2O_2$, indicating that these pulse exposures activate a stress response in potato tuber cells that may be similar to the rapid, $H_2O_2$-mediated, oxidative cross-linking of cell wall proteins [2] that precedes longer-term defensive responses to wounding or environmental stress [3]. To identify changes in plant cell wall porosity (cross-linking of polymeric components of the cell wall) after exposure to millisecond, kilovolt-per-meter pulsed electric fields, which may be used for electrotransformation, electropermeabilization, and for the electroporative introduction of preservatives and other compounds into fruits, vegetables, and other food materials.

**Methods.** Potatos (*Solanum tuberosum* cv. white rose) from a local market (Los Angeles, CA, USA) were manually washed, peeled, and sliced to obtain a rectangular core 1.5 cm long and 7.0 mm thick oriented perpendicular to the major tuber axis. Sections (7 mm x 7 mm x 1 mm) were cut from the phloem parenchyma tissue of the slice, rinsed with distilled water, and used immediately. Experimental samples were treated with 25 mM $H_2O_2$ in 5 mM KCl for 5 min, or pulsed after incubation in 10 mM sodium azide in 5 mM KCl for 15 minutes, or pulsed after incubation in 5 mM KCl. Electric pulses were delivered to tissue sections placed between two parallel, flat, stainless steel electrodes separated by 7 mm using a pulse generator designed and assembled in the department of Electrical Engineering, University of Southern California. Samples were exposed to a single 1 ms pulse at a range of applied field strengths (3, 10, 20, 30, 40, and 50 kV/m) and placed in 2 $\mu$M FM1-43 (Molecular Probes) in 5 mM KCl for 2 minutes before microscopic examination with a Zeiss Axiovert 200 epifluorescence microscope with a 10X objective. Images were captured and analyzed with a Zeiss camera (AxioCam MRm) and software (AxioVision 3.1).

**Results.** The rate of FM1-43 staining of potato tuber cell membranes, a function of the rate of diffusion of the dye through the cell well matrix [4], is greatly reduced after incubation of the potato sample in $H_2O_2$, which decreases cell wall porosity by inducing oxidative cross-linking of cell wall proteins [5]. H2O2 alone does not affect FM1-43 fluorescence. Within 30 seconds after exposure of potato tissue to single, 1 ms electric pulses in the range of 20 to 50 kV/m we see a marked reduction in the FM1-43 staining rate, which we interpret as an increase in cell wall cross-linking. No effect was observed with pulsed fields of 3 and 10 kV/m. Diffusion of FM1-43 in the extracellular space after electropulsation is not affected by pre-treatment with sodium azide, a strong peroxidase inhibitor [5,6].

**Conclusions.** Millisecond, kilovolt-per-meter electric pulses stimulate cell wall-associated peroxidase production of $H_2O_2$ in potato tuber tissue, with an associate decrease in cell wall porosity, consistent with the rapid burst of $H_2O_2$ production in plant cells after elicitor treatment [7,8] or in response to a wound [6].

1. Schote, U., and Seelig, J. 1998. Interaction of the neuronal marker dye FM1-43 with lipid membranes. Thermodynamics and lipid ordering. Biochimica et Biophysica Acta 1415, 135-46.
2. Apostol, I., Heinstein, P.F. and Low, P. 1989. Rapid stimulation of an oxidative burst during elicitation of cultures plant cells. Plant Physiology 90, 109-116.
3. Bradley, D.J., Kjelbom, P., and Lamb, C.J. 1992. Elicitor-and wound-induced oxidative

cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. Cell 70: 21-30.
4. Bolte, S., Talbot, C., Boutte, Y, Catrice, O, Read, N.D. and Satiat-Jeunemaitre, B. 2004. FM-dyes as experimental probes for dissecting vesicle trafficking in living plant cells. Journal of Microscopy 214, 159-173.
5. Bestwick, C.S., Brown, I.R., Bennett, M.H.R. and Mansfield, J.W. 1997. Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to Pseudomonas syringae pv phaseolicola. Plant Cell 9: 209-221.
6. Razem, F.A. and Bernards. M.A. 2003. Reactive oxygen species production in association with suberization: evidence for a NADPH-dependent oxidase. Journal of Experimental Botany 54: 935-941.
7. Brisson, L.F., Tenhaken, R. and Lamb, C. 1994. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. Plant Cell 6, 1703-1712.
8. Bolwell, G.P., Davies, D.R., Gerrish, C., Chung-Kyoon, A. and Murphy, T.M. 1998. Comparative biochemistry of the oxidative burst produced by rose and French bean cells reveals two distinct mechanisms. Plant Physiology 116, 1379-1385.

**Acknowledgements.** Supported by the Portuguese Foundation of Science and Technology, the Swedish Foundation for International Cooperation on Research and Higher Education, the Air Force Office of Scientific Research, and MOSIS.