Nemchinov LG, Shabala L and Shabala S. 2007. **Calcium Efflux as a Component of Hypersensitive Response of *Nicotiana benthamiana* to *Pseudomonas syringae***. Plant Cell Phys. E-pub Dec 3. 2007.

In this work, we studied mechanisms underlying hypersensitive response (HR) – a form of programmed cell death in plants associated with resistance responses to incompatible host-pathogen interactions. Ion fluxes across cellular membranes play a key role in triggering and mediating such defense mechanisms. Using a model plant *Nicotiana benthamiana* and incompatible, HR-causing strain of plant-pathogenic bacterium *P. syringae* pv *syringae* 61 (Pss 61, **Fig. 1**), we have demonstrated that initial calcium uptake in response to Pss 61 is followed by the net calcium efflux initiated at about 12 hours after the bacterial challenge and sustained for at least 48 hours. We suggest that this pattern of calcium signaling may indicate that *P. syringae*-induced HR is a multi-step process and consists of several phases. An initial calcium entry into the cells is required for the induction of basal defense response and activation of HR, while the late calcium efflux could potentially reflect its functional role in the development of a more expanded cell death and completion of defense reaction (**Fig. 2**).

A B C

Figure 1. Hypersensitive response (HR) on leaves of *Nicotiana benthamiana* plants inoculated with *Pseudomonas syringae* pv *syringae* 61. **A**, mock-inoculated plants; **B**, plants inoculated with *Pseudomonas syringae* pv *syringae* 61; **C,** plants inoculated with virulent strain *Pseudomonas syringae* pv *tabaci*.

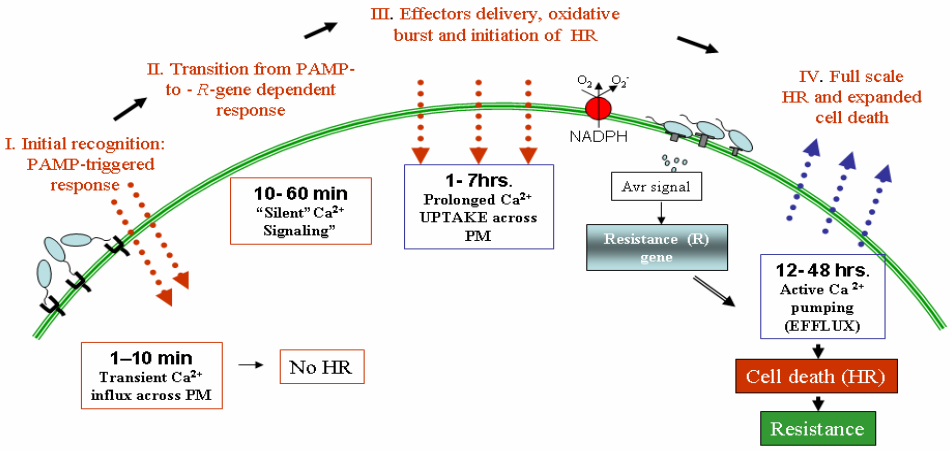

Figure 2. A proposed timing of calcium ion fluxes in mediation of plant defense responses. PAMP, pathogen-associated molecular patterns; HR, hypersensitive response; PM, plasma membrane; NADPH, nicotinamide adenine dinucleotide phosphate oxidase - mediated production of Reactive Oxygen Species (ROS) in the form of O_2^-.