

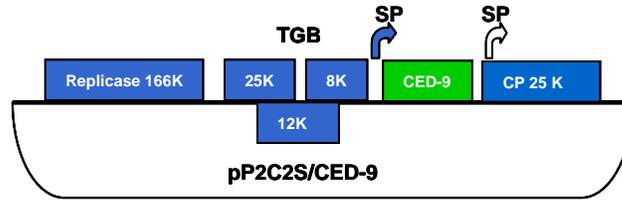
Virus-based expression of anti-apoptotic gene in plants modifies plasma membrane ion fluxes in response to salinity and oxidative stress

Apoptosis, one form of programmed cell death (PCD), plays an important role in mediating plant adaptive responses to the environment. Recent studies suggest that expression of animal anti-apoptotic genes in transgenic plants may be an efficient way of enhancing stress resistance in economically important crops. However, the underlying cellular mechanisms of this process and apoptotic signaling pathway in plant cells remain largely unexplored.

This work was aimed at addressing the above issue. Unlike previous studies, we used transient expression of anti-apoptotic gene CED-9 from nematode *Caenorhabditis elegans* by means of a plant-virus-based vector, PVX (Figure 1A). The benefits of virus-based transient gene expression versus transgenic plants include rapid engineering, high levels of desired protein and its systemic spread in plant tissues within a short period of time.

The recombinant PVX/CED-9 virus was infectious and caused characteristic PVX symptoms that appeared on leaves of inoculated *N. benthamiana* plants 7-10 days post-inoculation and subsequently developed into a systemic infection (Figure 1B). The engineered virus remained stable and produced CED-9 protein for at least one month after inoculation. RT-PCR using both CED-9 specific- and PVX-derived primers confirmed the presence of target CED-9 RNA in plants as well as the recombinant nature of the PVX vector *in vivo* (Figure 1C). The amplified fragments of the expected size were repeatedly obtained from different *N. benthamiana* plants infected with PVX/CED-9 and the identity of RT-PCR product was verified by nucleotide sequencing. Western blots probed with CED-9-specific antibody confirmed a presence of the CED-9 protein product in PVX/CED-9 plants: the revealed protein bands corresponded to the predicted molecular mass (Figure 1D).

We further demonstrated using a range of electrophysiological techniques that expression of CED-9 anti-apoptotic gene in tobacco increased salt and oxidative stress tolerance by altering ion flux patterns across the plasma membrane. Our data showed that PVX/CED-9 plants were capable of preventing stress-induced K^+ efflux from mesophyll cells, so maintaining intracellular K^+ homeostasis. We attributed these effects to the ability of CED-9 to control at least two types of K^+ - permeable channels: outward-rectifying depolarization-activating K^+ channels (KOR) and non-selective cation channels (NSCC), (Figure 2). Discovered pattern of ion signaling may bring to light new facts not only on the mechanism of improved stress tolerance but also on the regulatory elements of PCD immediately affected by the expression of anti-apoptotic genes.



A

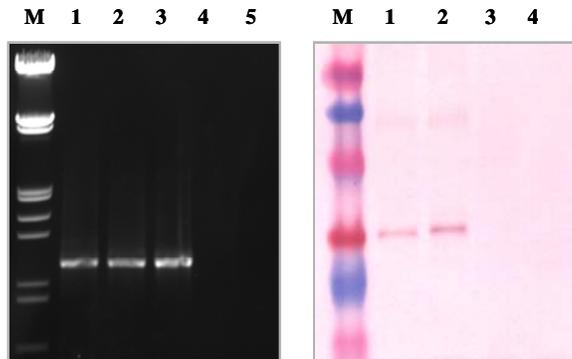


Control

PVX WT

PVX/CE9-9

B



C

D

FIGURE 1

A, A diagram of PVX/CE9-9 vector. **B**, Characteristic symptoms of wild-type PVX (WT) and PVX/CE9-9 on leaves of *N. benthamiana* plants. **C**, RT-PCR products amplified from plants infected with PVX/CE9-9 virus. **D**, Western blot analysis; membranes were probed with an antibody to CED-9.

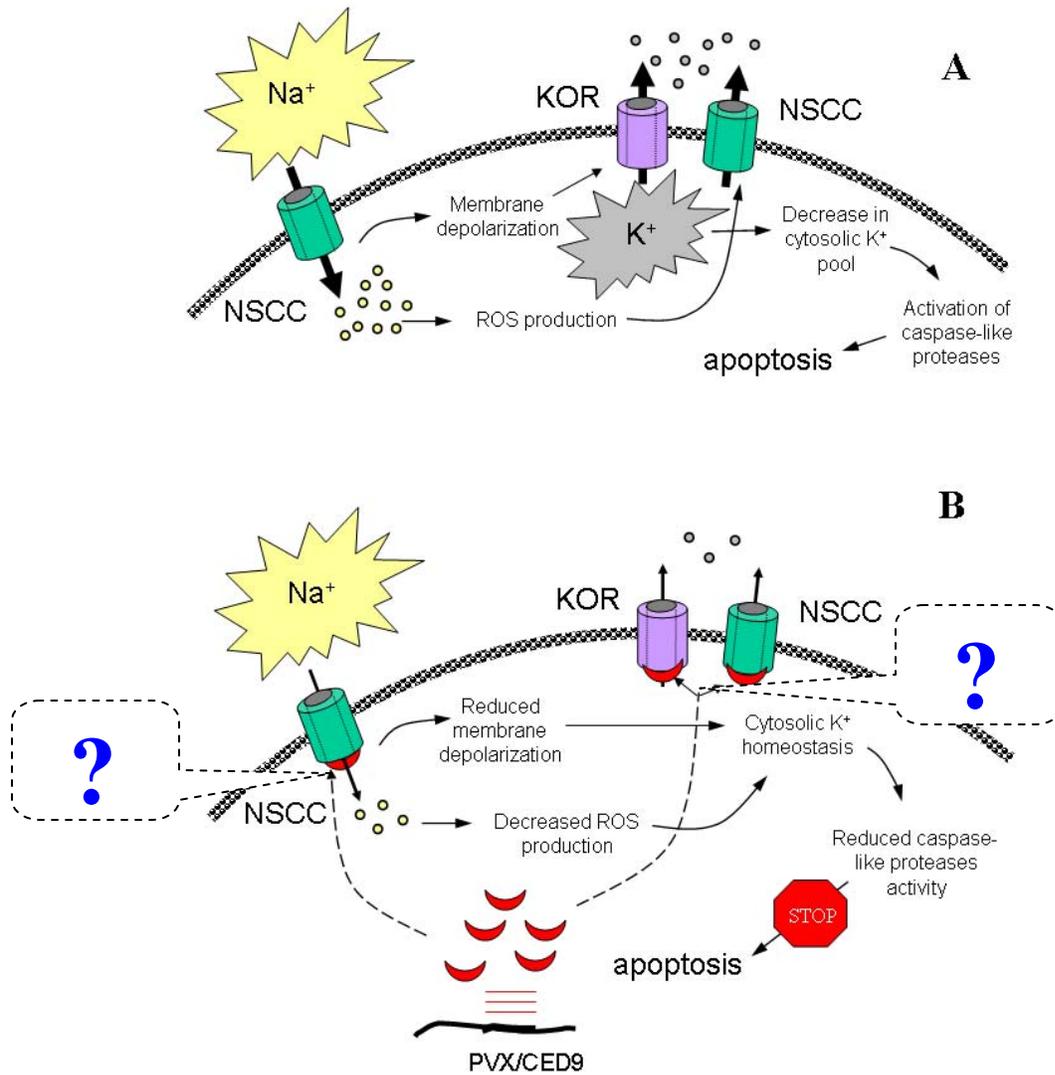


FIGURE 2

Proposed pattern of ion signaling initiated by transient expression of CED-9 in plants undergoing salinity stress. **A**, control plants under saline conditions. **B**, plants expressing CED-9 under saline conditions

Sergey Shabala, Tracey A. Cuin, Luke Prismall, and Lev G. Nemchinov. 2007. Expression of animal CED-9 anti-apoptotic gene in tobacco modifies plasma membrane ion fluxes in response to salinity and oxidative stress. *Planta*, published online on August 22, 2007. DOI 10.1007/s00425-007-0606-z