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Bax-induced cell death of *Arabidopsis* is meditated through reactive oxygen-dependent and -independent processes

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An *Arabidopsis* protoplast system was developed for dissecting plant cell death in individual cells. Bax, a mammalian pro-apoptotic member of the Bcl-2 family, induces apoptotic-like cell death in *Arabidopsis*. Bax accumulation in *Arabidopsis* mesophyll protoplasts expressing murine *Bax* cDNA from a glucocorticoid-inducible promoter results in cytological characteristics of apoptosis, namely DNA fragmentation, increased vacuolation, and loss of plasma membrane integrity. *In vivo* targeting analysis monitored using jellyfish green fluorescent protein (GFP) reporter indicated full-length Bax was localized to the mitochondria, as it does in animal cells. Deletion of the carboxyl-terminal transmembrane domain of Bax completely abolished targeting to mitochondria. Bax expression was followed by reactive oxygen species (ROS) accumulation. Treatment of protoplasts with the antioxidant N-acetyl-L-cysteine (NAC) during induction of Bax expression strongly suppressed Bax-mediated ROS production and the cell death phenotype.However, some population of the ROS depleted cells still induced cell death, indicating that there is a process that Bax-mediated plant cell death is independent of ROS accumulation. Accordingly, suppression of Bax-mediated plant cell deathalso takes place in two different processes. Overexpression of a key redox-regulator, *Arabidopsis* nucleoside diphosphate kinase 2 (AtNDPK2) down-regulated ROS accumulation and suppressed Bax-mediated cell death and transient expression of *Arabidopsis* Bax inhibitor-1 (AtBI-1) substantially suppressed Bax-induced cell death without altering cellular ROS level. Taken together, our results collectively suggest that the Bax-mediated cell death and its suppression in plants is mediated by ROS-dependent and -independent processes.