

ABSTRACT:

Zea mays is generally considered to be a plant with inducible lysigenous aerenchyma formation. The degradation of some cortical cells is triggered by environmental conditions, usually in the form of stress (submergence etc.). These cells die in a process that shows signs characteristic for programmed cell death, such as nuclear DNA fragmentation or apoptotic ultrastructural alterations.

Aerenchyma formed in primary roots of thirteen examined maize accessions, irrespective of cultivation conditions. The aerenchyma fraction correlated with the root length, not with its age. The dependence of aerenchyma formation on the presence of this phytohormone was proved by using an inhibitor of ethylene synthesis (AOA). It was found out that the aerenchyma formation depended on light conditions and that the lysigenous intercellular spaces colocalized with areas with cells with characteristically fragmented nuclear DNA (TUNEL-positive nuclei).

In experiments using the TUNEL reaction it was necessary to determine new dilution of the enzymatic mixture for the examined plant material. Only the observation of surface planes of free-hand root sections was considered relevant in both TUNEL-TMR and TUNEL-AP assays. TUNEL-AP was evaluated as a mixture with a worse penetrability. However, it (treatment "AP") could be observed under UV radiation without a risk of false positives caused by the autofluorescence of TUNEL-negative nuclei, which was (unlike the fluorescence of other nuclei) covered with a black product of the reaction of the alkaline phosphatase).

In addition to the TUNEL reaction, other procedures, generally used as cell viability methods, were applied to identify the cortical regions in which the cells started to die. The application of neutral red turned out to be rather unsuitable for the highly differentiated tissue of plant roots. The Evans blue labelled cells are prone to occur in some parts of the cortex – primarily in the vicinity of preformed aerenchyma channels, areas surrounding the protruding root primordia and in these newly emerging roots themselves.

Similarly localized, enhanced by the vascular tissue in the central cylinder, were cells labelled with fluorescent probes (PI, FDA, SYTOX, AO).

Based on the observations described, comparison with former results of TEM and the resemblance of these data with events accompanying differentiation of TEs known from literature, a putative sequence of events occurring during PCD in maize cortical cells during lysigenous aerenchyma formation was proposed:

In the cells designed for autolysis spherical vesicles containing esterases appear. These vesicles might be analogy of ricinosomes, transporting their contents to special targets, e.g. to the vacuole or cell walls, where they are later used for degradation processes. Later, the tonoplast loses its integrity and the central vacuole shrinks or breaks into several smaller vacuoles. The cytoplasm mixes with the cell sap and its pH decreases. Finally, the plasma membrane ruptures, the whole protoplast collapses and smaller plasmalemma bound vesicles form, in which the protoplast fragments with organelles including the nucleus are digested. The process is terminated with the cell walls disintegrating.

The course of PCD in cortical cells studied in wetland plants with lysigenous type of aerenchyma resembles processes described in the roots of maize in many respects, including nuclear DNA fragmentation, formation of vesicles with low pH containing esterases, tonoplast rupture, blending of cytoplasm and vacuolar contents and changes of plasmalemma permeability. Cells in which these changes take place are localized in the vicinity of emerging primordia, in idioblasts and in positions of future lysigenous aerenchyma. Their pattern corresponds to the symplastic connections described in literature.