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Short Communication

Apoptosis-Like Cell Death in Barley Roots under Salt Stress

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Salt stress-induced cell death was investigated in barley roots. Cleavage of nuclear DNA was observed 1 h after salt stress. Oligonucleosomal fragments of DNA were detected electrophoretically 8 h after salt stress. These phenomena indicate that apoptosis-like cell death can occur under salt stress.

Key words: Apoptosis-like cell death — Barley — DNA fragmentation — Salt stress — TUNEL.

Under salt stress condition, elongation growth of the plant cell is inhibited because high osmotic pressure of the external medium reduces cell turgor and inhibits the water uptake by the roots. Also important in the mechanism of cellular damage induced by salt stress, especially at a higher concentration of NaCl, is ionic toxicity. Katsuhara and Kawasaki (1996) reported that salt stress induced DNA degradation and cell death in barley root tips.

This short communication reports the fragmentation of DNA under salt stress. In order to detect the cleavage of nuclear DNA in the early stage of cellular injury, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) was applied to barley roots.

Barley (*Hordeum vulgare* L., cv. Akashinriki) was cultured hydroponically as described previously (Katsuhara and Kawasaki 1996). Salt stress was applied to seedlings (4-d old) by addition of 500 mM NaCl to the hydroponic solution. The concentration of $CaCl_2$ was fixed at 1 mM.

For electrophoretic analysis, DNA was isolated from the 10- to 15-mm-long root tips including root caps, as described previously (Katsuhara and Kawasaki 1996). For electrophoretic analysis, ladders of DNA fragments, which differed by less than 200 bp, were detected from 8 h after salt stress (Fig. 1). Low molecular DNA was hardly detected on the agarose gel 4 h after salt stress. The ladder structure represents an increase in oligonucleosomal fragments of DNA produced by endonuclease breakage in the linker regions between nucleosomal cores (Cohen 1993). These phenomena in the early stage of cellular injury are thought to result in nuclear degradation and cell death in the later stage (Katsuhara and Kawasaki 1996). This ladder structure became unclear 24 h after salt stress, suggesting random digestion of DNA strands.

For micrographic investigation, root tips were harvested, fixed, and dehydrated as described previously (Katsuhara and Kawasaki 1996). Samples were embedded in Technovit 8100 (Kulzer, Germany) supplemented with 5% (v/v) butoxyethanol (Furukawa et al. 1994) to enhance the permeation of TdT into the sample. Nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole) (Katsuhara and Kawasaki 1996) and fluorescein-dUTP (In Situ Cell Death Detection Kit, Fluorescein, Boehringer Mannheim, Germany) simultaneously, and observed by fluorescence microscopy (Olympus BH2). Fluorescein-dUTP was incorporated into the breakage of the DNA strand by TdT and showed yellow fluorescence. Total DNA was observed with blue fluorescence stained by DAPI.

In order to detect the cleavage of nuclear DNA, TUNEL, which was introduced to study the cell death of animal cells, has recently been applied to plant systems

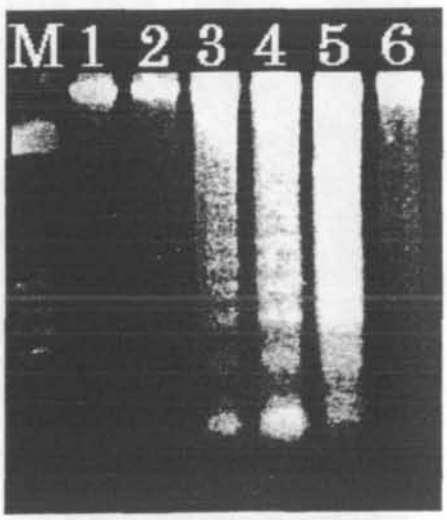

Fig. 1 Formation of oligonucleosomal fragments of DNA with salt stress. M, 100-bp ladder marker; Lane 1, DNA isolated from control roots (0 h). DNA isolated from roots subjected to stress for 4 h, Lane 2; 8 h, Lane 3; 12 h, Lane 4; 24 h, Lane 5. Lane 6, DNA isolated from roots with control treatment (without salt stress) for 24 h. Each lane was loaded with 2 μg DNA.

Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; TdT, terminal deoxynucleotidyl transferase; TUNEL, TdT-mediated fluorescein-dUTP nick end labeling.

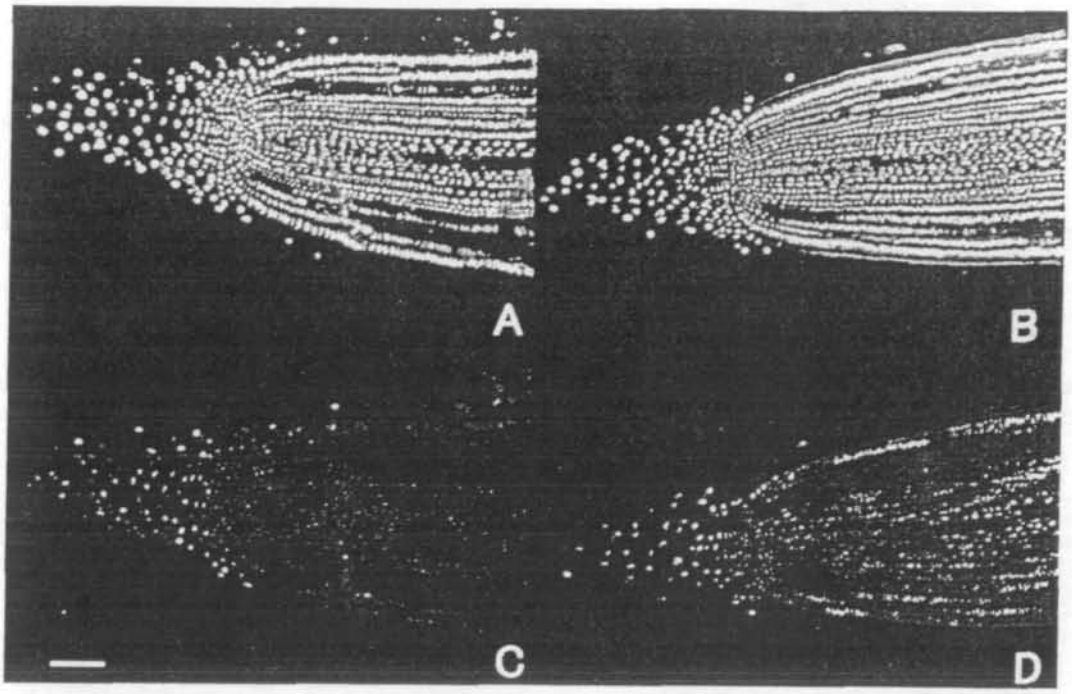

Fig. 2 Longitudinal sections of roots. A control root (A and C) and a root subjected to salt stress for 1 h (B and D) were simultaneously stained with DAPI (A and B) and TUNEL (C and D). Bar shows 100 μm.

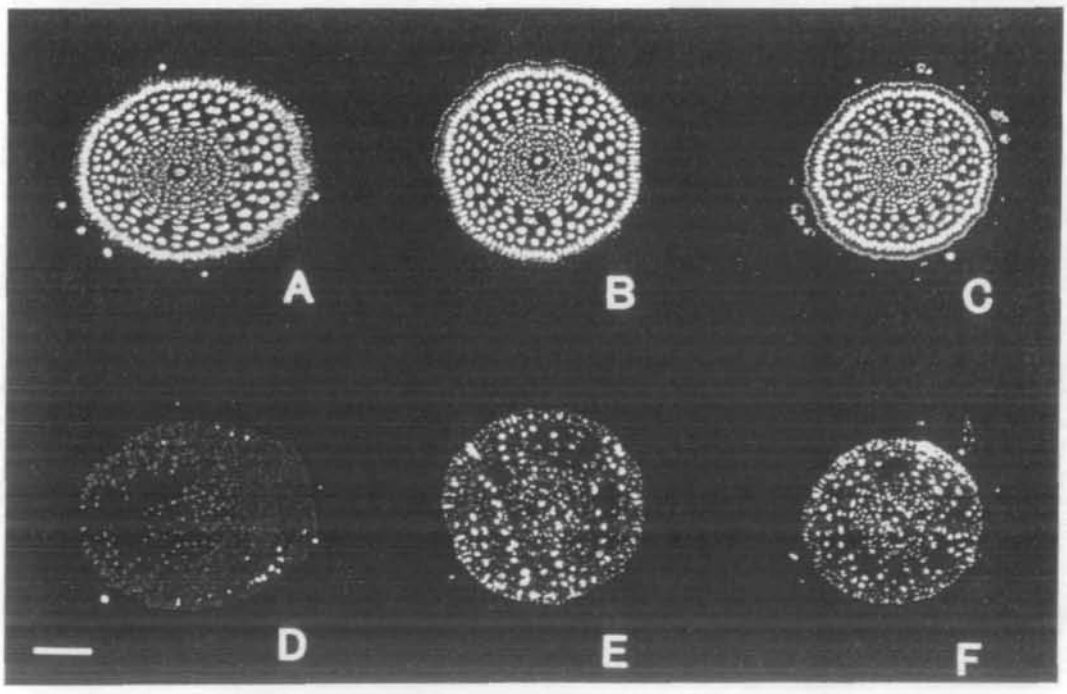

Fig. 3 Cross sections of roots at around 200 μm from the root apical meristem. A control root (A and D), a root subjected to salt stress for 1 h (B and E), and a root with salt stress for 4 h (C and F) were simultaneously stained with DAPI (A, B, and C) on TUNEL (D, E, and F). Bar shows 100 μm.

(Mittler and Lam 1995, Ryerson and Heath 1996, Wang et al. 1996). In the present study of barley roots, only nuclei in cells of the control root cap fluoresced a brilliant yellow (TUNEL-positive), indicating that DNA breakage occurred only in the root cap (Fig. 2).

After salt stress, however, most nuclei in the root became TUNEL-positive within 1 h. No difference was observed in the fluorescence from fluorescein-dUTP between cells in the outer layer and those in the inner layer (Fig. 3). Brightness and the percentage of TUNEL-positive nuclei did not change between cells treated with salt stress for 1 and 4 h (Fig. 3).

A TUNEL-positive character is recognized as one feature of apoptosis, a type of cell death defined morphologically and biochemically in animal cells. However, apoptosis is also characterized by other features, such as a ladder structure of DNA on agarose gel, cell shrinkage, and the formation of apoptotic bodies. In barley root under salt stress, only some of the apoptotic features (TUNEL-positive character and the ladder structure of DNA) were observed. Thus, cell death observed in this study was tentatively termed as apoptosis-like cell death. It should be pointed out again that a TUNEL-positive character principally indicates an increase in the 3'-OH of DNA strands and that the ladder structure of DNA represents the DNA cleavage between nucleosomes. In animal cells, the involvement of these biochemical events in apoptosis is well-established, but this is debatable for plant cells. Further studies are required to verify the validity of applying the term "apoptosis" to plant cells.

Previously, the mechanism of cell death in plants has been investigated as a reaction to pathogens (Ryerson and Heath 1996, Wang et al. 1996), or as cell differentiation (xylogenesis; Mittler and Lam 1995, Fukuda 1996). The present study includes no information on cell death in the region where xylogenesis occurs (at 3 or more mm from the root apex in barley, Moritsugu et al. 1993), because the focus was only on cells in the immature region (within 2 mm from the root apex). Together with previous findings (Katsuhara and Kawasaki 1996), this communication demonstrated that salt stress can trigger cell death with some apoptosis-related features.

The question then arises of whether cell death under salt stress has some physiological implications. This cell death may separate the disordered root system from plant tissue and may prevent the influx of excess ions into the shoot. Another possibility is that DNA degradation produces nucleotides (and the putative degradation of protein may produce amino acids), and that these materials may be relocated and recycled for shoot and/or new root formation when salt stress is temporary or adjustable at the seedling level. Work is under way to test these hypotheses.

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