Quantitative Analysis of Cell Division and Cell Death in Seminal Root of Rye under Salt Stress

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Abstract : Cell division and cell death in the cell division zone of the roots of rye seedlings under salt stress were analyzed quantitatively. Cell division was examined by immunological staining with anti-5-bromo-2'-deoxyuridine (BrdU) and cell death by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL). In the 0-700 μm portion from the root tip, which is the cell division zone, the frequency of cell division increased linearly during BrdU treatment. Therefore, the frequency of cell division under salt stress was compared with that in the control at 7 hours after application of BrdU. In the 250 mM NaCl solution (salt stress), the frequency of cell division was decreased and that of cell death was increased as compared with the control resulting in the inhibition of root elongation. In the presence of 50 and 100 mM NaCl, the frequency of cell division was also significantly increased and cell death was hardly detected, and root growth was unchanged as compared with the control. These results suggested that the increase of cell division complemented the decrease of cell elongation due to salt stress, and consequently maintained root growth under mild salt stress conditions.

Key words : Cell death, Cell division, Root elongation, Rye (*Secale cereale* L.), Salt stress.

The plant root system plays an important role in the uptake of water and nutrients, which are supplied to the shoot. The plant organ first exposed to salt stress is the root. The growth of primary roots and development of lateral roots are affected differently by salt stress (Rubinigg et al., 2004). When the seedlings of *Arabidopsis* were transferred to the media with various concentrations of NaCl, the growth rate of the primary roots was reduced with increasing NaCl concentration (West et al., 2004). Salt stress inhibits the extension growth of primary roots in many plants, e.g., in *Arabidopsis* (West et al., 2004), cotton (Kurth et al., 1986; Reinhardt and Rost, 1995) and maize (Rodrígues et al., 1997). It also inhibits the growth of lateral root in cotton (Reinhardt and Rost, 1995). However, production and growth of lateral roots were less affected by salt stress in radish (Waisel and Breckle, 1987), or even stimulated by salt stress in *Plantago maritima* (Rubinigg et al., 2004).

Root elongation includes both cell elongation and cell production. There are many studies on the relationship between cell elongation and osmotic stress brought about by either drought or salinity stress, and it is considered that the turgor pressure and mechanical properties of cell wall affect the elongation and growth of cells (Lockhart, 1965). Frensch and Hsiao (1994) monitored the change of elongation in the root elongation zone, the loss of turgor pressure and recovery of root growth under osmotic stress in maize seedlings. For the turgor maintenance in the elongating zone of seminal roots in maize seedlings under osmotic stress, osmotic adjustment plays an important role (Sharp et al., 1988, 1990; Voetberg and Sharp, 1991; Rodrígues et al., 1997; Verslues and Sharp, 1999; Ogawa and Yamauchi, 2006a, b). The cell-wall extensibility was decreased by salt stress in maize root (Neumann et al., 1994).

On the other hand, the effect of salt stress on cell production is not well understood. The change of cell production due to salt stress has been shown by indirect indicators, i.e., cell size (Kurth et al., 1986), enzyme activities (West et al., 2004) and expression of genes related with the cell cycle (Burssens et al., 2000). However, there are no reports directly evaluating the change in the cell production ability under salt stress. The frequency of cell division determines the rate of cell production. For quantitation of the frequency of cell division, the ratio of the number of cells with newly replicated DNA to that of all cells should be measured. Immunological staining with anti-5-bromo-2'-deoxyuridine (BrdU) is available for the detection of the cells with newly replicated DNA. BrdU is an analogue of thymidine and can be incorporated into DNA in place of thymidine when the cell division occurs. Cells which have incorporated BrdU into DNA can be quickly detected using a monoclonal antibody against BrdU and an enzyme- or fluorochrome-conjugated second antibody. This method has some advantages; i.e., higher resolution and sensitivity, and easier experimental procedures (Suzuki et al., 1992) than microautoradiography with ^{3}H-thymidine (Fujie et al., 1993b). Since Gratzner (1982) used a monoclonal antibody against BrdU for the detection of DNA replication, this method has been used for the
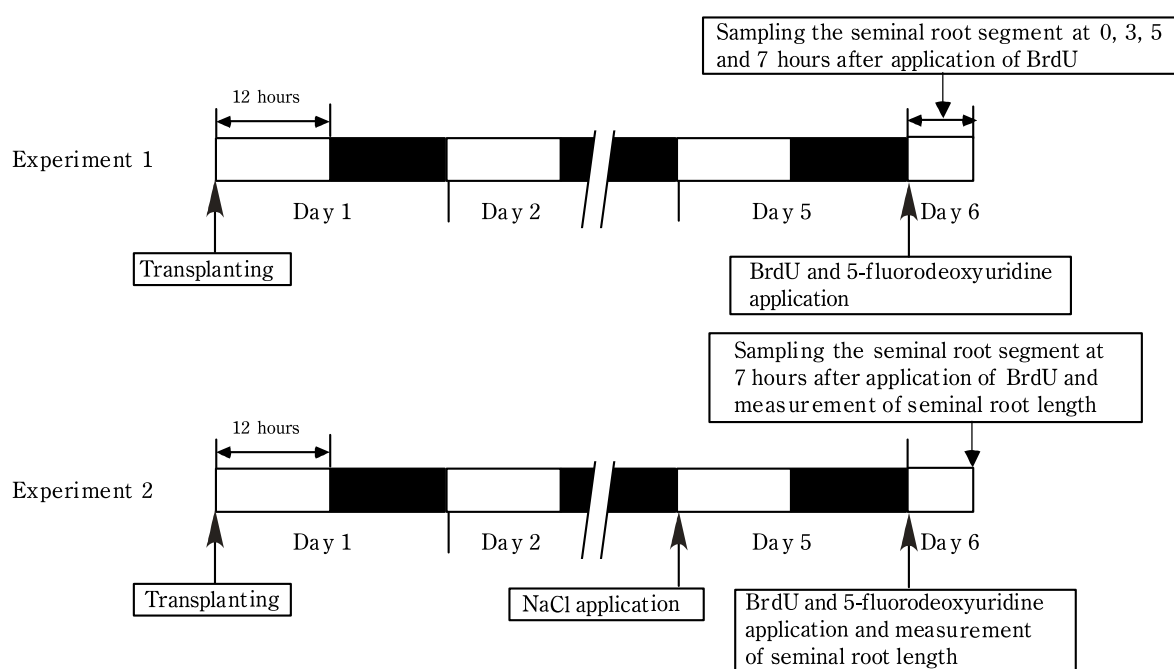
Fig. 1.   Schedule of Experiment 1 and Experiment 2. Closed bars show dark period and open bar light period.

detection of DNA replication in animals and plants. In plants, DNA replication has been detected in cultured cells of tobacco (Suzuki et al., 1992), root tips of *Arabidopsis thaliana* (Fujie et al., 1993a) and *Allium cepa* (Hervás et al., 2002), and the excised leaf of tobacco (Stroobants et al., 1990).

Moreover, it is considered that some cells in the cell division zone die under salt stress. In plants as in animals, there are many studies on programmed cell death termed apoptosis (e.g. Lam et al., 2000 for review). Apart from programmed cell death, environmental stress also causes apoptosis-like cell death. Under salt stress, cell death caused by cleavage of nuclear DNA was observed. Katsuhara and Kawasaki (1996) reported that seminal root growth of barley was inhibited by 200 mM NaCl. They examined the nuclei of meristematic cells in the roots under salt stress by staining with DAPI, and observed nuclear deformation of cells after 12-hour treatment with 500 mM NaCl, and nuclear degradation after a 24-hour treatment with NaCl above 300 mM. Furthermore, aluminum-induced cell death in the root of barley (Pan et al., 2001), cytotoxic reagent-induced cell death in maize roots (Ning et al., 2001), and mechanical stress-induced and nitric oxide-induced cell death in *Arabidopisis* leaves (Garces et al., 2001) have also been reported.

In the early stage of cellular injury before apoptosis-like cell death, DNA is cleaved to 180–200bp fragments (Katsuhara and Kawasaki, 1996). Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) is available for the *in situ*

detection of DNA fragmentation. In this method, fluorescein-12-dUTP is incorporated into the 3´-OH DNA ends using the terminal deoxynucleotidyl transferase, recombinant, (rTdT) enzyme. The fluorescein-12-dUTP-labeled DNA, in which DNA fragmentation occurred, can be visualized directly by fluorescence microscopy. Katsuhara (1997) reported that DNA fragmentation in the seminal root of barley occurred after one hour of 500 mM NaCl treatment, and the TUNEL-labeled cells were increased with increasing treatment time. However, they did not evaluate quantitatively the effect of cell death on the root growth.

The objective of this study was to examine the effect of cell division and cell death in the cell division zone on the root growth under salt stress in rye seedlings. Rye is classified as a moderately salt tolerant plant from the growth of shoot under salt stress condition, and the salt tolerance is lower than barley and wheat and is higher than maize and rice among gramineous crops (Mass, 1987; Jumberi et al., 2001). In this study, the frequency of the cell division and that of the cell death were quantitated using immunofluorescence microscopy against BrdU and TUNEL, respectively, for the examination of the effect of salt stress on the rye root growth.

Materials and Methods

1. Plant materials

Seeds of rye (*Secale cereale* L., Snow Brand Seed Co., Ltd.) were germinated in the dark at 28°C in petri dishes for 3 days. During this time, the seminal root

elongated approximately 10 mm in length. These seedlings were transplanted onto nets floating in a one-liter beaker filled with nutrient solution. Twenty seedlings were planted in one beaker. The nutrient solution contained 6.0 mM KNO_3, 4.0 mM $Ca(NO_3)_2$, 1.0 mM $NH_3H_2PO_4$, 2.0 mM $MgSO_4$, 26.8 μM Fe-EDTA, 4.6 μM $MnCl_2$, 23.1 μM H_3BO_3, 0.38 μM $ZnSO_4$, 0.16 μM $CuSO_4$, and 0.015 μM $(NH_4)_6Mo_7O_{24}$. The nets were covered with aluminum foil with pinholes to allow roots to grow in the dark. The nutrient solution was aerated sufficiently. The plants were grown under a 12-hour photoperiod in a growth chamber (MLR-350H, SANYO). The photon flux density of photosynthetically activity radiation (PAR, 400-700 nm) was 320 μmol m^{-2} s^{-1}. The chamber was maintained at 20ºC with 80% relative humidity during the day and night. Seedlings were transplanted at the onset of light time (Fig. 1).
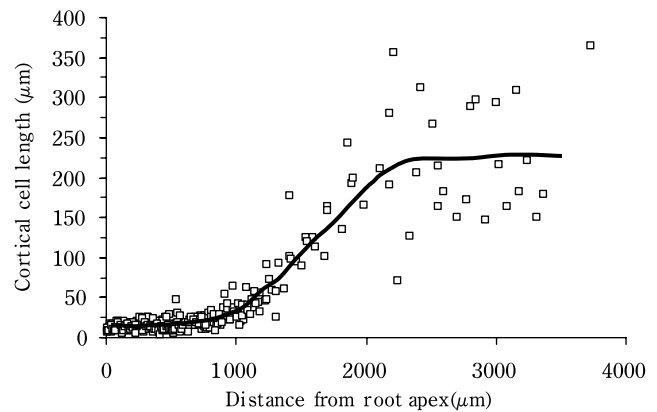

Fig. 2. Cortical cell length of seminal root sampled at the 7th hour after application of BrdU and 5-fluorodeoxyuridine as a function of the distance from the root apex. Three root segments were used for the measurement.

2. Experiment 1: Time required for BrdU-labeling

Five days after transfer of the plants to the net, 1 mL of 10 mM BrdU solution and 1 mL of 1 mM 5-fluorodeoxyuridine solution were applied to the 1L nutrient solution; the resulting final concentrations were 10 μM and 1 μM, respectively (Fig. 1). 5-Fluorodeoxyuridine is the synthetic inhibiter of thymidine (Fujie et al., 1993a).

After the application of these solutions, segments of seminal root approximately 5-mm in length were sampled from the root tip at 0, 3, 5, 7-hour (Fig. 1), fixed with 4% (w/v) paraformaldehyde in PBS buffer (pH 7.4), dehydrated with a graded ethanol series and embedded in Technovit 7100 (Okenshoji Co., Ltd.). Longitudinal sections were made at 2 μm thickness using a microtome (HM360, Microm), and sections were dried at 37ºC. Samples were collected with five replications.

The cell division zone was determined in the sections prepared from samples obtained at the 7th hour rehydrated with 10 mM phosphate buffer (PBS; 2.68 mM KCl, 1.45 mM Na_2HPO_4, and 8.6 mM KH_2PO_4), stained with Meyer Heamatoxylin (Sakura Finetek Japan Co., Ltd.) for 1 hour, washed with water for 5 minutes, dried at 37ºC, and mounted with Eukitt (EMS). After drying at 37ºC, stained material was observed under a microscope (BX51, Olympus) with a digital camera (Camedia C-5050, Olympus). The image data of cortex cells in the second layer was captured basipetally from the tip, and each cell length was calculated from the image data using Lumina Vision OL Vl.11 (Mitani Corporation). The cell division zone was determined from the data of 318 cells in three seminal roots.

The 5-bromo-2´-deoxyuridine Labeling and Detection Kit I (Cat. No. 11 296 736 001, Roche Applied Science) was used to detect the DNA-replicating zone and to measure the frequency of cell

division. The sections were rehydrated by washing three times with PBS each for 5 minutes, and dried to stain the peripheral zone. The sections were covered with a sufficient amount of anti-BrdU working solution [anti-BrdU with nucleases (mouse monoclonal antibody containing nucleases for DNA denaturation) diluted 1:10 in incubation buffer (66 mM Tris-buffer, 0.66 mM $MgCl_2$, 1 mM 2-mercaptoethanol)], and incubated for 1 hour at 37ºC in the humid box. They were rinsed three times each for 5 minutes with PBS, dried to stain the peripheral zone, covered with anti-mouse-Ig-fluorescein working solution [anti-mouse-Ig-fluorescein stock solution (anti-mouse-Ig-fluorescein solution was dissolved in 1 ml distilled water) was diluted 1:10 in PBS] and incubated for 1 hour at 37ºC in the humid box. The sections were vigorously washed with PBS three times each for 5 minutes, then washed with PBS containing 0.1% Triton X-100 and 5 mg/ml BSA for 5 min, and finally with PBS for 5 minutes. For counterstaining, they were covered with 4', 6-doamidino-2-phenylindole dihydrochloride n-hydrate (DAPI) diluted to 2.5 μg/ml in PBS with 1% Triton X-100 for 20 minutes at room temperature in the dark. They were rinsed with PBS for five minutes, mounted with ProLong Gold Antifade Reagent (Molecular), and dried at 37ºC for the microscopic observation. For the negative control, anti-BrdU with nucleases was omitted from the anti-BrdU working solution.

3. Experiment 2: Effect of salt stress on the cell division and cell death

After 4-day culture of the plants on the net, 0 (control), 0.58, 2.92, 5.84 and 14.61 g per liter of NaCl were added to the nutrient solution to adjust the NaCl concentration to 0, 10, 50, 100 and 250 mM, respectively (Fig. 1). After 24-hour culture in the NaCl solutions, 1 mL of 10 mM BrdU solution and 1 mL of 1 mM 5-fluorodeoxyuridine solution were added to the 1
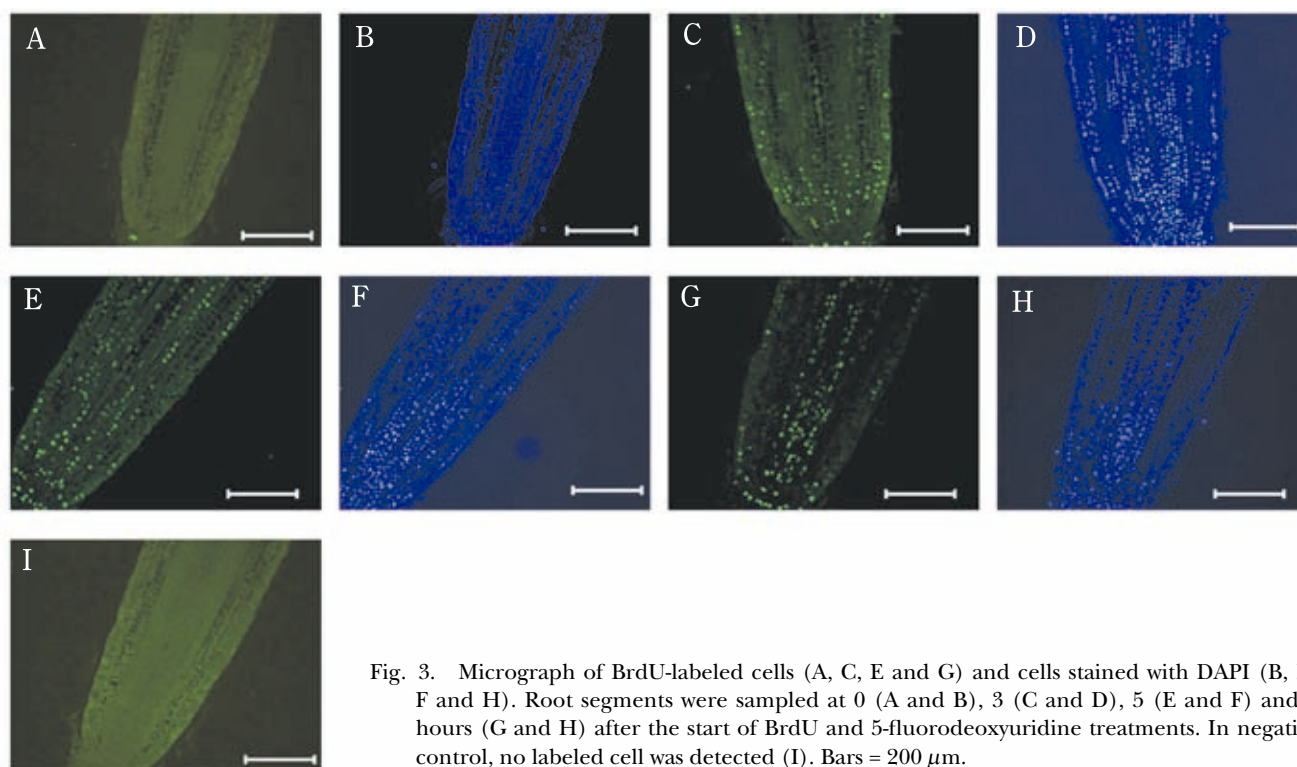
Fig. 3. Micrograph of BrdU-labeled cells (A, C, E and G) and cells stained with DAPI (B, D, F and H). Root segments were sampled at 0 (A and B), 3 (C and D), 5 (E and F) and 7 hours (G and H) after the start of BrdU and 5-fluorodeoxyuridine treatments. In negative control, no labeled cell was detected (I). Bars = 200 μm.

L nutrient solution (final concentrations were 10 and 1 μM, respectively) to examine the frequency of cell division.

At the 7th hour, segments of seminal root with approximately 5 mm length from the root tip were sampled, and longitudinal sections were made as described in Experiment 1. The samples were collected with five replications.

The DeadEnd™ Fluorometric TUNEL System (G3250, Promega) was used for the detection of DNA fragmentation and measurement of the frequency of the cell death. The sections were rehydrated for five minutes with and PBS containing 0.85% NaCl. They were then fixed with 4% (w/v) paraformaldehyde in PBS for 15 minutes, rinsed twice with PBS each for 5 minutes, and dried to stain the peripheral zone. Proteinase K solution [20 μg Proteinase K was dissolved in Proteinase K buffer (100 mM Tris-HCl, 50 mM EDTA)] was dropped on the samples, and they were incubated at room temperature for 10 minutes. Then they were rinsed for five minutes with PBS, fixed with 4% (w/v) paraformaldehyde in PBS for 5 minutes, and rinsed for 5 minutes with PBS. Excess liquid was removed, and equilibration buffer (200 mM potassium cacodylate, 25 mM Tris-HCl, 0.2 mM DTT, 0.25 mg/ml BSA, 2.5 mM cobalt chloride) was dropped on the sample and incubated for 10 minutes. After that, rTdT incubation buffer [equilibration buffer, Nucleotide Mix (50 μM fluorescein-12-dUTP, 100 μM dATP, 10 mM Tris-HCl, 1 mM EDTA) and rTdT enzyme were mixed at a ratio of 45:5:1] was dropped
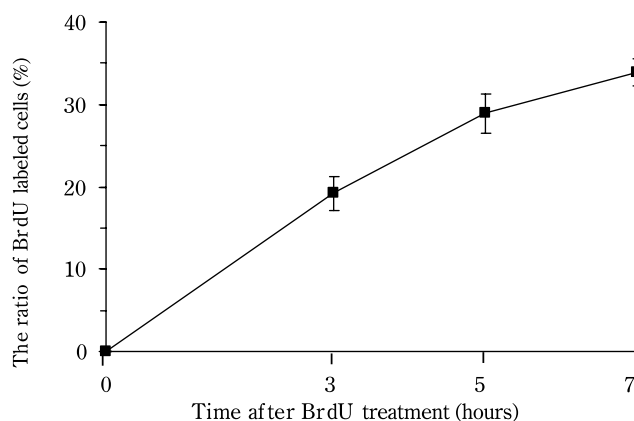

Fig. 4. Change of the ratio of BrdU-labeled cells to total cells (stained with DAPI) during for 7 hours of BrdU and 5-fluorodeoxyuridine treatments. Data are means $\pm$ standard errors (n=5).

on the samples, and they were incubated at 37ºC for 60 minutes in a humid box in the dark. After incubation, the samples were treated with 2 × SSC for 15 minutes followed by three 5-minute washing with PBS containing 0.1% Triton X-100 and 5 mg/ml BSA and a 5-minute wash with PBS. For counterstaining, they were incubated in PBS containing 2.5 μg DAPI/ml and 1% Triton X-100 in the dark at room temperature for 20 minutes. They were rinsed with PBS for 5 minutes, mounted with ProLong Gold Antifade Reagent (Molecular), and dried at 37ºC for the microscopic observation. For the negative control, rTdT Enzyme

was omitted from the rTdT incubation buffer. For the positive control, the samples were treated with DNase I buffer (40 mM Tris-HCl, 10 mM NaCl, 6 mM MgCl$_2$, 10 mM CaCl$_2$) containing 10 unit/ml of DNase I for 10 minutes at room temperature before incubation with equilibration buffer.

The DNA-replicating cells were detected as described in Experiment 1.

We also examined the effect of salt stress on root elongation. The seedlings with the average length of seminal roots were selected, and the seminal root length was measured before and after the stress treatment. Five replications were used for the measurement of seminal root length.

4. The microscopic observation to detect DNA-replicating cells and dead cells

The sample stained as above was observed with an epi-fluorescence microscope (BX51, Olympus) with a charge coupled device (CCD) camera (P70-WPCXP, Olympus) and image data were captured using a DP Controller (Olympus). BrdU and TUNEL-labeled nuclei were observed with a WIB filter (excitation at 495nm; emission > 520 nm) and DAPI-labeled nuclei were observed with a WU filter (excitation at 372 nm; emission > 456 nm). The number of nuclei labeled with BrdU, TUNEL and DAPI was counted using Lumina Vision OL Vl.11.

The frequency of cell division was calculated by the following formula;

The frequency of the cell division（％）=

$$\frac{\text{The number of BrdU-labeled nuclear}}{\text{The number of DAPI-labeled nuclear}} \times 100$$

where BrdU-labeled nuclei show the cells under cell division (BrdU was incorporated into DNA), and DAPI-labeled nuclei are all nuclei on the section. The frequency of cell death was calculated by the following formula;

The frequency of the cell death（％）=

$$\frac{\text{The number of TUNEL-labeled nuclear}}{\text{The number of DAPI-labeled nuclear}} \times 100$$

where TUNEL-labeled nuclei show the cells undergoing DNA fragmentation, and DAPI-labeled nuclei are all nuclei on the section. Five replications were used for measurement of the frequency of cell division and frequency of cell death.

Results

1. Detection of cell division zone

Cell length was stable at 0-900 μm portion from the root tip, cell elongation started from the 900 μm portion, and then cell length increased basipetally (Fig.
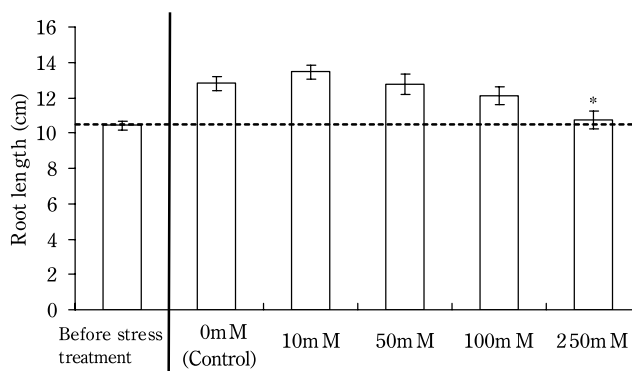

Fig. 5.　Effect of 31-hour salt stress on the seminal root elongation. Data are means ± standard errors (n=5). * indicates significant differences by a t-test at P<0.05 compared with the control values.

2). At about 2500 μm, cell elongation stopped, and cell length attained a constant value of approximately 250 μm. Based on this result, we defined the 0-700 μm portion from the root tip as the cell division zone and observed cell division and cell death in this zone.

2. Experiment 1: Time required for BrdU labeling

The BrdU-labeled cells, which show DNA-replicating cells, were observed 3, 5 and 7 hours after the application of BrdU and 5-fluorodeoxiuridine. No signal of BrdU labeling was observed before the treatment with BrdU and 5-fluorodeoxyuridine (Fig. 3-A). DNA replicated occurred uniformly in all regions of the cell division zone. The degree of BrdU-labeling was the highest at the 7th hour (Fig. 3-G), followed by the 5th and the 3rd hour in this order. In the negative control, no labeled cells were detected (Fig. 3-I).

Fig. 4 shows the ratio of the number of BrdU-labeled cells (Fig. 3-A, 3-C, 3-E and 3-G) to the number of all cells stained with DAPI (Fig. 3-B, 3-D, 3-F and 3-H) on the section. The ratio increased almost linearly for 7 hours after application of BrdU; the frequency was 19.2 % at the 3rd hour, 28.9 % at the 5th hour and 33.9 % at the 7th hour. This means that cell division occurred throughout the 7 hours after BrdU application, and that cell division was not inhibited by BrdU incorporation until the 7th hour. In Experiment 2, therefore, the frequency of cell division under salt stress was examined at the 7th hour after BrdU application.

3. Experiment 2: Effect of salt stress on the cell division and cell death

The effect of salt stress on the elongation of seminal root was investigated (Fig. 5). The seminal roots treated with 0, 10, 50 and 100 mM NaCl elongated 2.4, 3.0, 2.4 and 1.7 cm, respectively, during 31 hours after the start of the treatment. Thus, NaCl at 10-100 mM did not affect the root elongation. However,
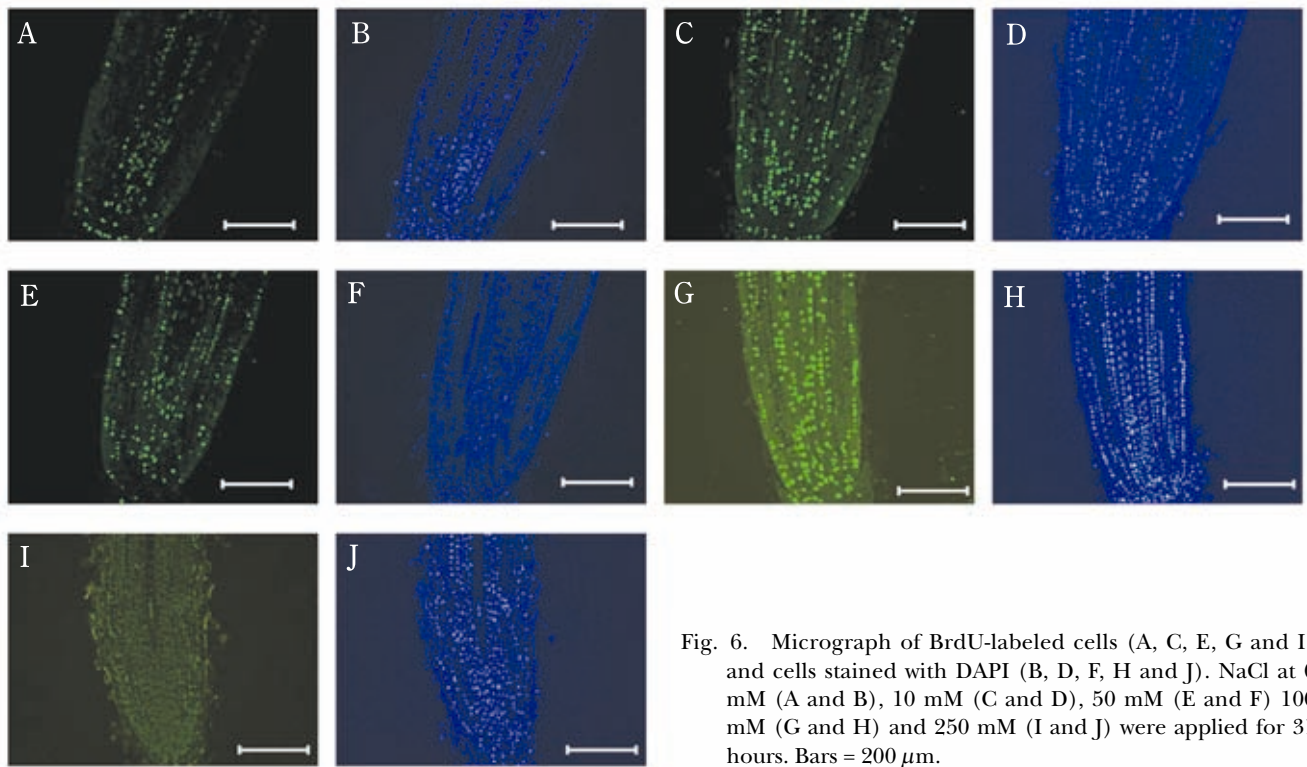
Fig. 6. Micrograph of BrdU-labeled cells (A, C, E, G and I) and cells stained with DAPI (B, D, F, H and J). NaCl at 0 mM (A and B), 10 mM (C and D), 50 mM (E and F) 100 mM (G and H) and 250 mM (I and J) were applied for 31 hours. Bars = 200 μm.

the seminal roots treated with 250 mM NaCl for 31 hours elongated only 0.3 cm. There was no significant difference between the root lengths before and after 31-hour treatment with 250 mM NaCl.

Fig. 6 shows the micrographs of the cells labeled with BrdU for 7 hours under salt stress. BrdU was incorporated into the cells exposed to 10 mM (Fig. 6-C), 50 mM (Fig. 6-E) and 100 mM (Fig. 6-G) NaCl as in the control (Fig. 6-A). On the other hand, BrdU-labeled cells were hardly detected in the cells exposed to 250 mM NaCl (Fig. 6-I).

The frequency of cell division in the cell division zone under salt stress was calculated from the ratio of the number of BrdU-labeled cells to that of all cells stained with DAPI on the section (Fig. 7). The frequency of the cell division in 10 mM NaCl was 39.1 %, which was not significantly different from that in the control (34.0 %). In 50 mM and 100 mM NaCl, the frequency of cell division was 45.3 % and 42.3 %, respectively, which were significantly higher than that in the control (P<0.05). On the other hand, in 250 mM NaCl, the frequency was 1.87 %, which was significantly lower than that in the control (P<0.001). These results showed that the cell division was promoted by 50 mM and 100 mM NaCl and was inhibited in 250 mM NaCl compared with the control.

Fig. 8 shows the micrographs of the cells labeled with TUNEL, which show DNA fragmentation in nuclei in the cell division zone of seminal root under salt stress. TUNEL-labeled cells were hardly detected after 31 hours in 10 mM (Fig. 8-C), 50 mM (Fig. 8-E) and
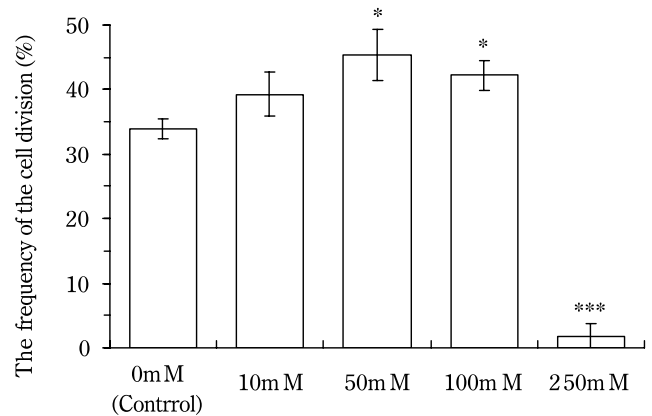

Fig. 7. Effect of salt stress on the frequency of cell division, respectively by BrdU-labeling. Data are means ± standard errors (n=5). * and *** indicates significant differences by a t-test at P<0.05 and P<0.001 compared with the control values, respectively.

100 mM (Fig. 8-G) NaCl as in the control (Fig. 8-A). On the other hand, in 250 mM NaCl, many TUNEL-labeled cells were detected, and DNA fragmentation was seen in most cells (Fig. 8-I). In the negative control, no labeled cells were detected (Fig. 8-K), and most cells were labeled in the positive control (Fig. 8-L).

The frequency of cell death in the cell division zone under salt stress was calculated from the ratio of the number of TUNEL-labeled cells to the number of all cells stained with DAPI on the section (Fig. 9). The
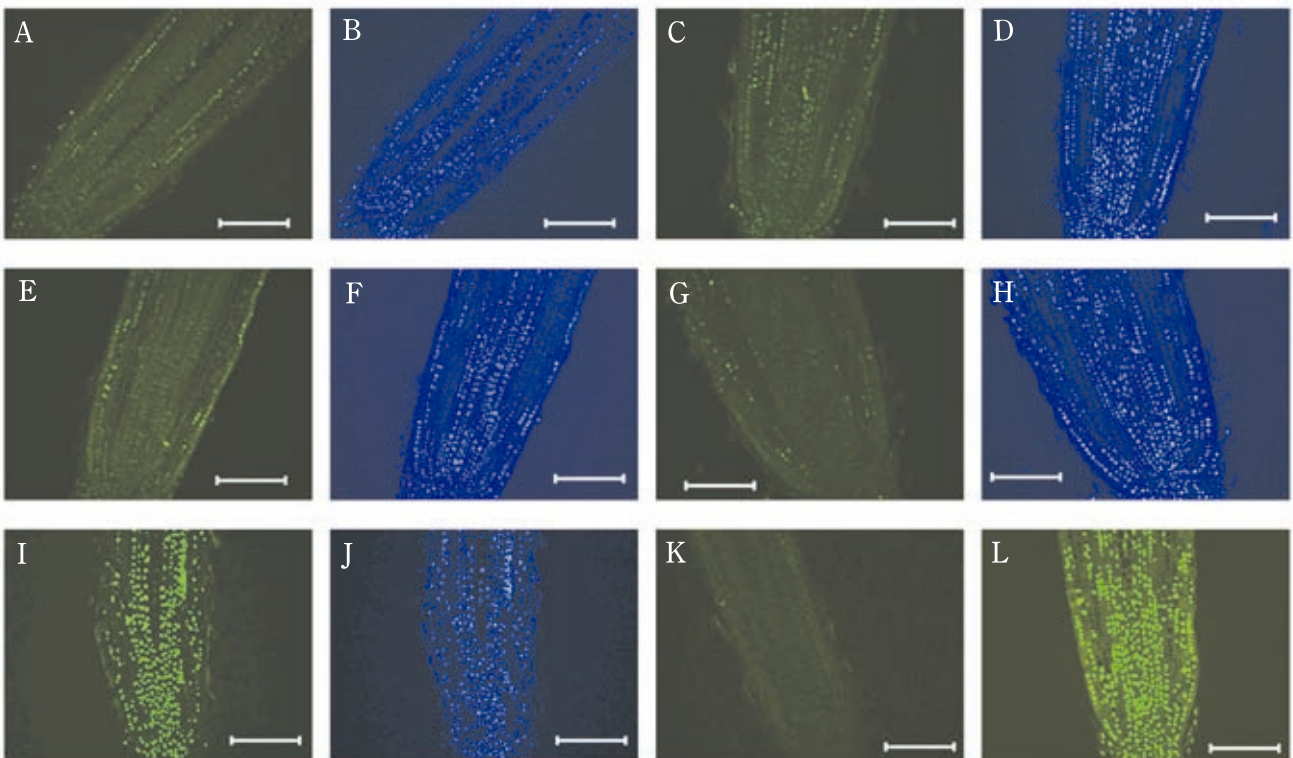
Fig. 8. Micrograph of TUNEL-labeled cells (A, C, E, G and I) and cells stained with DAPI (B, D, F, H and J). The roots were treated with 0 mM (A and B), 10 mM (C and D), 50 mM (E and F) 100 mM (G and H) and 250 mM (I and J) NaCl for 31 hours. In negative control, no labeled cells were detected (K), and most cells was labeled in positive control (L). Bars = 200 μm.

frequency of cell death was very low in the control (4.21 %). Similarly, the frequency was low in 10 mM (1.02 %), 50 mM (4.30 %), and 100 mM (2.93 %) NaCl and significant differences were not detected in the frequency of cell death compared with the control. On the other hand in 250 mM treatment, the frequency of cell death was 85.9 %, which was significantly different from the value in the control (P<0.001). These results showed that most cells were alive in the solution of NaCl at 100 mM or lower concentration and most cells died in the 250 mM NaCl solution.

Discussion

In this study, we showed that the cell division and cell death in the cell division zone affected root growth under salt stress in rye seedlings. In this study, the frequency of the cell division and the frequency of cell death in the cell division zone were quantitated using immunofluorescence microscopy against BrdU and TUNEL, respectively.

Since BrdU is incorporated into DNA during cell division, it is predicted that the proportion of BrdU-labeled cells would increase with the application time of BrdU in the portion where cell division occurred constantly. In the 0-700 μm portion from the root tip, the frequency of BrdU-labeled cells increased almost linearly for 7 hours after BrdU application (Fig. 4).
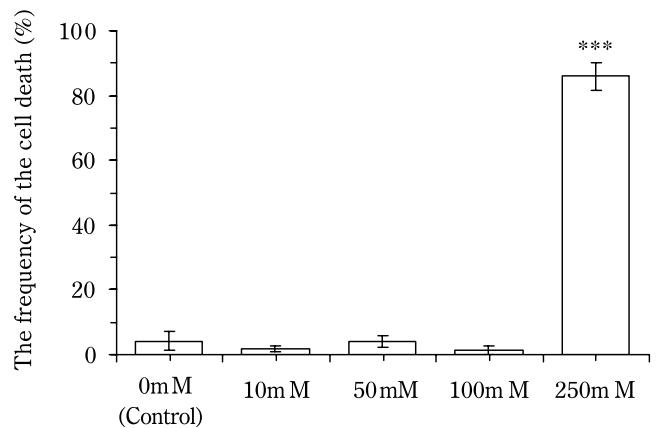

Fig. 9. Effect of salt stress on the frequency of cell death estimated by TUNEL labeling. Data are means $\pm$ standard errors (n=5). *** indicates significant differences by a t-test at P<0.001 compared with the control values.

The incorporation of ^{3}H-thymidine continued for 20 hours in the root apical meristem of *Arabidopsis thaliana* (Fujie et al., 1993b). In this study, the incorporation of BrdU was continued constantly until 7-hours after BrdU application, and cell division was not inhibited in the presence of BrdU for 7 hours (Fig. 4). From this result, we compared the value of the frequency of cell division under salt stress with that in

the control at 7 hours after BrdU.

Seminal root elongation was maintained in 10, 50 and 100 mM NaCl, but was inhibited by 250 mM NaCl (Fig 5). The cell division estimated by BrdU labeling was also inhibited by 250 mM NaCl, to 5.5% of the control level (Figs. 6 and 7). In previous studies, the effect of salt stress on the cell production was examined based on the change of the cell size, the activity of enzymes and the expression of genes related with the cell cycle. Kurth et al. (1986) examined the change of the growth rate and cell length in cotton roots under salt stress, and showed that the rate of cell production was decreased as the concentration of NaCl was increased to above 50 mM. West et al. (2004) examined the change of primary root elongation and cell size of the root in *Arabidopsis* under salt stress, and showed that growth reduction was induced by the decrease in cell production and smaller mature cell length. They also showed that the cyclin-dependent-kinase activity, which catalyzed the cell cycle progression, and the promoter activity of the mitotic cyclin *CYCB1;2* were transiently reduced after salt stress treatment in primary root of *Arabidopsis*. Burssens et al. (2000) examined the effect of expression of cell cycle regulatory genes under salt stress on the root growth in *Arabidopsis* and showed that the transcript level of the *CDC2aAt* gene, which encodes a cyclin-dependant kinase, was decreased along with the inhibition of root growth. Moreover, we observed that the frequency of the cell death in 250 mM NaCl solution was 21 times higher than that in the control (Figs. 8 and 9). Katsuhara and Kawasaki (1996) observed nuclear deformation in the cells after a 12 hour treatment with 500 mM NaCl, and nuclear degradation after a 24-hour treatment with NaCl above 300 mM. Katsuhara (1997) also observed TUNEL-labeled cells in the seminal root of barley after a one-hour treatment with 500 mM NaCl. In this study, we showed that seminal root elongation was inhibited by 250 mM NaCl treatment with the reduction of the frequency of cell division and the increase of frequency of the cell death. It followed from these result that the change of cell production affected root elongation under salt stress.

Cell length was decreased by salt stress in cotton (kurth et al., 1986), barley (Huang and van Steveninck, 1990) and *Arabidopsis* (West et al., 2004). In cotton root, the length of cortical cells declined as the concentration of NaCl was increased above 50 mM (kurth et al., 1986). In 150 mM NaCl, the cell length was about 20% of the control. Similarly in the primary root of *Arabidopsis,* the length of cells in the boundary between the meristem and mature zone cells and in the mature zone in 0.5 % NaCl treatment were 57 % and 76 % of control cells, respectively (West et al., 2004). In this study, we showed that the frequency of cell division was increased (Fig. 7) and the cell death was hardly detected in 50 mM and 100 mM

NaCl treatments (Fig. 9), though root elongation was not different from that in the control (Fig. 5). These results suggested that the increase of the frequency of cell division under mild salt stress of up to 100 mM NaCl complemented the decrease of longitudinal cell size, and consequently root elongation was maintained under mild salt stress.

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