Dynamics of Root Border Cells in Rhizosphere Soil of Zea mays L.:
Crushed Cells during Root Penetration, Survival in Soil,
and Long Term Soil Compaction Effect

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Abstract : Plant roots release mucilage and root border cells (RBCs) into rhizosphere, which function as a complex at the root-soil interface. The dynamics of RBCs in rhizosphere soil, however, remains unknown. In this study, the ratio of crushed root cap cells during root penetration into soil and survival of the RBCs after the release from the root cap were estimated in maize seminal root. In addition, the effects of long term soil compaction on RBCs release were investigated. During the root penetration into rhizosphere soil, 78, 56, and 45% of sloughed root cap cells were estimated to be crushed at the first, second, and third day after planting, respectively. The number of surviving RBCs decreased with time, but 6% of the RBCs in the rhizosphere still retained their cell walls at one month after planting. These cells were estimated to remain in the soil for at least 10 d after the release from lateral roots. Furthermore, RBCs release from newly emerged nodal root increased with aging of plants, and the cell release was significantly increased by soil compaction only at the seedling stage. In conclusion, significant number of RBCs were crushed during root penetration into soil, however many RBCs remained in the rhizosphere soil for a relatively longer period. Soil compaction significantly increased cell release only at the seedling stage.

Key words : Detached cells, Maize, Rhizosphere, Root cap, Sloughed root cap cells, Soil compaction, Soil mechanical impedance.

The plant root cap is known to release high molecular weight polysaccharide mucilage and thousands of root border cells (RBCs) into rhizosphere soil (Wen et al., 2007; Iijima et al., 2008). RBCs are traditionally considered as dead cells detached from the root cap (Driouich et al., 2007). RBCs, however, play both physical and biological roles at the root-soil interface in the rhizosphere soil. One of the most important functions is to reduce the soil frictional resistance during root penetration into soil (Iijima et al., 2000; 2003b; 2004a; 2004b). In agricultural field soils, the majority of RBCs will be crushed and/or partly broken by abrasion with soil particles during root penetration into the narrow soil pore space. Some of the released intact cells are also partly broken during the separation process from the soil particles for the quantification of RBCs (Iijima et al., 2004b). Hence, we can not clearly distinguish between the group of cells released as intact cells and those partly broken. In this context, we referred to these partly broken root cap cells found in rhizosphere soil as ‘root border cells’ (RBCs) which exists in root-soil interface. Although extensive studies on RBCs have been carried out, the dynamics of RBCs released from the plants grown in a real soil condition has not been studied fully so far. How many RBCs were initially produced and disappeared by root penetration? How long do the RBCs survive in soil? This study was designed to answer these questions on the dynamics of RBCs.

The cells produced in the root cap meristem are pushed forward as new cells form beneath them, and eventually, the cells at the periphery of root cap fall off. The cell-wall structure in the group of cells in the outermost cap layers is open sparse and loose, and easily broken by mechanical abrasion (Iijima and Kono, 1992). Because the outermost cap cell layers were strongly pressed between root body and soil particles during root penetration into soil, some RBCs may even be completely crushed. As a result, a copious amount of cell debris adheres on both the epidermal cell surface near the root cap and soil particles that exist in the root-soil interface. The population of these crushed RBCs can be estimated by the quantification of cell production rates in the root cap meristem, changes in the cap cell number, and the number of RBCs release (Iijima et al., 2003a). Previously, we estimated that 46% of RBCs of young maize seminal roots were crushed during one-day penetration into sandy loam soil. No other experimental evidence has been reported on how many cells are crushed during

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Abbreviations : ANOVA, analysis of variance; DAP, days after planting; RBCs, root border cells.
root penetration into soil. The information on the ratio of crushed cell will give basic information on the studies of mechanical impedance of soil and plant root growth.

The period of existence of RBCs after release to rhizosphere soil is unknown. Available information is limited to the statement by Vermeer and McCully (1982). They stated that sloughed root cap cells in field-grown nodal roots of maize survive for at least a few days in rhizosphere soil without the quantitative analysis. The existence of these cells may affect the root function such as absorption of water and nutrients from soil, and interaction with soil microorganisms.

The number of RBCs released into rhizosphere soil increases as the soil penetration resistance increases (Iijima et al., 2000). The increased cell release in compact soil reduces soil frictional resistance during root penetration (Iijima et al., 2003b), and thus the root can penetrate into hard soil. The number of RBCs released in compact soil, however, was analyzed only in a few-day-old (only one day growth in soil) very young seedling roots. Question arises here that whether the number of RBCs release in much older plants also increase in compact soil to reduce frictional resistance. The higher cell release in compact soil may be only a temporal phenomenon during seedling establishment of crop species. In the present study, we analyzed the dynamics of RBCs; estimating the percentage of cells crushed by root penetration, the duration of RBCs existence in rhizosphere soil, and the effects of long term soil compaction on RBCs release to answer the questions raised above.

Materials and Methods

1. Seedlings and soil preparation

Maize (Z. mays L. cv. Robust 30–71) caryopses were soaked in distilled water for 30 min and placed on moistened blotting paper in petri dishes at 30ºC for 48 hr in the dark for germination. Germinated caryopses, each with a straight seminal root 20–35 mm in length, were used in all the experiments. Loamy sand soil (Kiso river alluvial soil; particle size distribution, sand 87.0%, silt 9.6%, clay 3.4%) sieved through a 2 mm mesh and mixed with powdered compound synthetic fertilizer (N: 12%, P₂O₅: 16%, K₂O: 14%) at the rate of 0.4 g kg⁻¹, was used in a series of experiments.

2. Separation of soil released RBCs from soil particles

Initially, seminal roots with soil particles adhered were placed in a 2 mL eppendorf tube and added with surfactant solution (mixture of Tween 80 and sodium diphosphate), as described previously (Iijima et al., 2004b). The eppendorf tube was gently shaken 3 times followed by 1 min sonication and left for 10 s. Thereafter, 0.8 mL of the supernatant solution was pipetted into another 2 mL eppendorf tube containing 0.2 mL surfactant solution, and centrifuged at 90 g for 20 s after a flash sonication. Centrifuged supernatant solution of 0.8 mL was discarded and topped with 790 μL surfactant solution and 10 μL Toluidine Blue O (3 × 10⁻⁵ g g⁻¹) and then gently rotated for 1 hr. The number of RBCs was directly counted under a light microscope at 400 × magnification. In all the experiments, mostly rounded and elongated cap cells were enumerated, since they generally correspond to cells that have originated from the columella and peripheral regions of the root cap, respectively (Guinel and McCully, 1987). Partly broken sloughed root cap cells with more than three quarters of cell walls of intact cells, were regarded as RBCs because they can be easily distinguished as cells originating from the root cap from their shape. Others were not counted due to the difficulty in distinguishing from other cell debris derived from epidermal cells.

3. Estimation of crushed RBCs (Experiment 1)

Germinated caryopses were transplanted to plastic pots (40 cm long and 5 cm in diameter) filled with loamy sand soil. Seminal roots of the plants were grown in rhizosphere cylinders (small tube of 18 cm long and 0.5 cm in diameter), whose bottom was open for free root elongation, fixed to the wall of the plastic pot (40 cm long, 5 cm in diameter) filled with loamy sand soil (Fig. 1). The percentage of RBCs crushed during root penetration into soil was estimated in the seedling root grown for up to 5 d with three–six replicates. The soil water content (gravimetric basis) was adjusted every day to 25% (Ψw=−7 kPa). The percentage of the crushed RBC population was estimated from the time course difference in total cell number in root cap, cap cell production rates per unit time, and RBCs release rate. Total cap cell number (N)
was estimated using eqn (1) based on volume of whole root cap (V) and each cell in the root cap (v). Root cap volume was calculated by the eqn (2) derived from the mathematical integration method by rotating the two-dimensional estimation of root cap with respect to its y axis (Fig. 2). Volume of cap cell was estimated using eqn (3) assuming that the cells were cylindrical in shape (Iijima et al., 2000).

\[ N = \frac{V}{v} \]  
\[ V = V_1 - V_2 = \int_0^{L_1} \pi X_1^2 dy - \int_0^{L_2} \pi X_2^2 dy \]  
\[ v = \frac{\pi (d/2)^2 l}{8} \]

Where D2 is the diameter of the apex of the root that lies under the cap; L1 is the length of the root cap; f1 and f2 are the functions of the curves of two dimensionally expressed root cap and root tip, respectively (Fig. 2); d and l are the diameter and length of cap cell, respectively. The number of RBCs released to rhizosphere soil were quantified by the method mentioned above on seminal root portions elongated within each 24 hr (Fig. 3 left). Finally, the number of crushed cell numbers were estimated based on total number of cells lost to the rhizosphere and the number of intact cells recovered at the rhizosphere.

4. Quantification of RBCs survival (Experiment 2)

Germinated caryopses were transplanted to plastic pots as described in experiment 1, filled with the same type of soil. The rhizosphere cylinder was used to continuously monitor the survival of RBCs released from same timing of plant age. As stated above, partly broken sloughed root cap cells which have more than three quarters of their cell walls of intact cells, were regarded as surviving RBCs because we can not distinguish whether this small breakage was caused by the separation process of RBCs from soil particles or not. Plants were grown at 20°C, under a 12 hr photoperiod with the photosynthetically active radiation of 175 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) and 70% relative humidity for one month where the soil water content (gravimetric basis) was adjusted every day to 25% (\( \Psi_w = -7 \) kPa). The plants were harvested at 1, 10, 20 and 30 d after planting (DAP). The existing RBCs were quantified in the root portion elongated during the first day (first-day-root portion) of the seminal root (Fig. 3 right) with 5–6 replicates. RBCs were enumerated according to the technique described above for the total number of survived RBCs. RBCs may be produced continuously from newly emerged lateral roots along the root axis. Therefore, a similar experiment was performed to find out the time course production of lateral roots emerged in the first-day-root portion to determine the age of the RBCs existing in the same root portion.
5. Effects of long-term soil compaction on RBCs production (Experiment 3)

Three germinated caryopses were planted at the center of each pot (30 cm long and 25 cm diameter) filled with loamy sand soil under two soil compaction levels, either, loose (1.30 Mg m$^{-3}$) or compact (1.50 Mg m$^{-3}$). After one week, the number of seedlings was reduced to one per pot. The experiment was conducted in a greenhouse with average minimum and maximum temperatures of 25 and 37ºC, respectively, and the soil water content was adjusted to field capacity of water by adding an adequate amount of water every one to two days. Four replicated samples were harvested at 2, 5 and 8 wk after planting. The root tips of newly emerged nodal roots, ranging from 20 to 50 mm in length were cut and immersed in distilled water for 3 hr. The root cap mucilage adhered tightly on the cap cells expanded fully by absorbing the surrounding water (Iijima et al., 2003c). The fully hydrated mucilage was transferred to a 2 mL eppendorf tube. Subsequently, 960 μL surfactant solution and 40 μL Toluidine Blue O (5×10$^{-5}$ g g$^{-1}$) were added and the number of RBCs was directly counted under a microscope after 1 hr of hydration.

6. Statistical analysis

Mean values of a given variable together with the SE of the mean were calculated. Differences between the soil compaction treatments were subjected to one-way analysis of variance (1-way ANOVA). Comparison of means was performed at a 5% probability level with Tukey’s LSD test.

Results

1. Estimation of crushed RBCs (Exp. 1)

RBCs crushed during root penetration into soil can be estimated from the change in cell number inside and outside the root cap. Cells remaining in the root cap were estimated by the root cap dimension (Table 1). In general, the length and diameter of the root cap decreased with time, and therefore, the estimated cap cell number decreased with time (Table 1). The numbers of rhizosphere RBCs in the root portion elongated each day gradually decreased from 1 to 5 DAP, when the emergence of lateral roots was not recorded (Table 2). RBCs survival during five days of short duration is also shown in Fig. 4. For example, in the first-day-root portion, the percentage of RBCs decreased gradually with time, i.e., 59%, 40% and 27% of RBCs survived at 2, 3 and 5 DAP, respectively. The second-day-root portion showed a similar trend, but with higher values, i.e., 69% and 52% survived after one to three days of leave in root-soil interface, respectively. For the quantitative analysis of cell crush rate (Table 3), the cap cell production rate was regarded as 1,570 cells per day, according to our previous study assuming that the rate is stable from 1 to 3 DAP (Iijima et al., 2003a). The environmental
conditions of plant growth such as growth temperature and soil bulk density were the same in both experiments. With reference to the estimated number of cells lost in rhizosphere and RBCs remained, 78, 56, and 45% of the released RBCs were crushed during root penetration at the first, second, and third day, respectively. The rates of crushed cells by abrasion with soil particles during root penetration reduced gradually with time.

2. Quantification of RBCs survival (Exp. 2)

The number of RBCs remained in the rhizosphere soil decreased with time (Fig. 5). At 1 DAP, an average of 1,021 RBCs were found and the number was reduced to 204, 111 and 58 at 10, 20 and 30 DAP, respectively. The quantified values of RBCs were the sum of surviving cells attached to the seminal root axis and the cells released from the cap of emerging lateral roots. Lateral roots were started to emerge at 6 DAP, but no more lateral roots were produced after 20 DAP in that region, hence at least 10 d of lateral root free period was existed. Therefore, the minimum longevity of RBCs was 10 d, since six percent of the total RBCs still remained at 30 DAP.

3. Effect of long term soil compaction on RBC production (Exp. 3)

The number of RBCs increased with aging of plants in both loose and compacted soil treatments (Fig. 6). Soil compaction increased the release of RBCs at the early growth stage. At 2 wk, the number of RBCs in compact soil was 2.3 times that in loose soil treatment (Fig. 6, $F_{1,20} = 15.1, P < 0.001$). At later growth stages, however, the difference was not statistically significant.

Discussion

The plant root cap is an important tissue to reduce the soil frictional resistance during root penetration into soil. In most of the ordinal root growth in soil except for the loosely prepared nursery bed or continuous soil cracks, the root has to overcome soil frictional resistance for root expansion in soil to acquire the scattered water and nutrient. During root penetration, some of the cells in the outermost layers of root cap will be crushed by abrasion with

| Days after planting (DAP) | Total cap cell number | Difference in cell number between succeeding DAP | Cell production rates (d⁻¹) † | Cells lost in rhizosphere (b + c) | Detached cap cells | Intact cells ‡ | Crushed cells (d–e) |
|--------------------------|-----------------------|-----------------------------------------------|-------------------------------|--------------------------------|-------------------|----------------|---------------------|
| 0                        | 9177                  |                                               |                               |                                |                   |                |                     |
| 1                        | 6376                  | 2601                                          | 1570                          | 4171                           | 918               | 3253 (78.0%)    |                     |
| 2                        | 5132                  | 1444                                          | 1570                          | 3014                           | 1336              | 1678 (55.7%)    |                     |
| 3                        | 3756                  | 1376                                          | 1570                          | 2946                           | 1620              | 1326 (45.0%)    |                     |

# See Table 1 for total cap cell number. † From Iijima et al. (2003a). ‡ See Table 2 for total intact RBCs.
soil particles. The crushed cells most probably reduce the soil resistance to root penetration. This study first estimated the changes in crushed cell rates. It decreased with time as indicated in Table 3. During the first day of root elongation, between 0 and 1 DAP, about four-fifths of the cap cells were crushed during the initial root penetration into soil. These cells should be easily crushed due to the nature of cell life in the root cap. The cell walls in the outermost cap cell layer are often sparse and not densely packed, and therefore, are easily decayed (see in electron micrograph of Iijima and Kono, 1992). After the initial (first day) root penetration, most of the loosely attached cells in outermost layers should be removed from the root cap, and the second to third layers of root cap will be exposed to the root-soil interface. Their cell walls should be much stronger than those in the outermost layer, since they are still at an immature stage. Therefore, the crushed rate would have decreased gradually as the days of root penetration increase. At 2 to 3 DAP, only half of the cells were crushed, which is similar to the estimation in our previous study of 0 to 1 DAP (Iijima et al., 2003a).

In the previous study, we used median longitudinal paraffin sections for the estimation of total cap cell number, while intact root cap was used for the estimation in the present study. Paraffin embedding preparation remove the loosely attached RBCs, which would cause the difference in the RBCs crush rate in the two studies.

The number of RBCs surviving after root penetration was shown in Fig. 4. The cell numbers in the second- and third-day root portion were greater than that in the first-day portion, which indicated that the longevity of these cells was longer. This is due to the fact that these cells were most probably derived from second to third cell layers of the root caps. These cells may be removed from the root cap before fully mature or degraded stage.

Our results also showed that RBCs, with mostly intact cell walls, remained for a longer duration in soil than those in the previous statement by Vermeer and McCully (1982). Six percent of the RBCs in the first-day-root portion survived in the rhizosphere at 30 DAP (Fig. 5). The longevity of RBCs remaining in this root portion is longer than 10 d since there was no lateral root production after 20 DAP. The RBCs remaining should be attached closely to the epidermis of seminal root axis, and may acquire materials essential for the survival in soil, or may directly acquire them from rhizosphere soil. These cells may affect rhizosphere microbial populations as a carbon source (Nguyen, 2005), or through secretion of mucilage (Hawes et al., 2000), export of an array of low molecular proteins (Zhu et al., 1997), and interaction with protozoan population (Somasundaram et al., 2008). They may also affect nutrient and water transport between root-soil interface, if RBCs remain in the soil pore space where plenty of soil water and nutrients are available. The viability of these surviving cells should be analyzed further to test the ability to influence the rhizosphere environment.

The effect of long term soil compaction was examined by the quantification of RBCs loosely attached to the newly emerged nodal root at different growth stages. Production of RBCs was not significantly different between the two compaction treatments in 5- and 8-wk old matured plants, although the compact treatment showed higher production than loose treatment at the seedling stage of 2-wk-old plants (Fig. 6). This means that the RBCs release increases as the plant age progressed in loose treatment. Although the loose treatment showed progressive increment of RBCs release, compaction did not show such a trend especially between 2 and 5 wk. Sloughing of cap cells from the mature plant should be fully analyzed to explain this phenomenon.

In summary, our results revealed that 78 to 45% of sloughed cap cells were immediately crushed by the root penetration into soil, and the surviving RBCs exist for up to 10 d. Furthermore, the soil compaction exerts its influence on RBCs release only at the early stage of plant growth. The proposed biological functions of RBCs such as, repelling or binding pathogenic bacteria (Hawes et al., 2000), synthesizing defensive structures in response to fungal attack (Sherwood, 1987) and producing a mucilage layer in response to co-cultivation with pathogenic bacteria (Hawes and Pueppke, 1987) are important when considering a soil environment, since we found that the RBCs can exist in soil for a long time after detachment. Most probably, the long term existence of RBCs was facilitated by receiving energy from the neighboring root hairs and/or epidermal cells, which need to be confirmed in future investigations. Furthermore, the prolonged existence of RBCs in the rhizosphere can act as a carbon source and gives beneficial effects on plant root growth and nutrient absorption through plant-microbe interaction. Therefore, the present findings will be valuable for analyzing the importance and involvement of RBCs, in a soil environment.

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