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Development of Schizogenous and Lysigenous Aerenchyma in Rice Root

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ABSTRACT

Aerenchyma development in rice (*Oryza sativa* L.) roots is quite important for adaptation to waterlogged or reduced soil conditions. Anatomical observations were carried out to clarify the development of schizogenous and lysigenous aerenchyma in elongating crown roots of rice. The crown roots of 3rd and 4th phytomer were taken from rice plants of the 8th leaf stage grown by hydroponic culture. The schizogenous intercellular spaces in the cortex of crown root tip were observed using a light microscope with semi ultra-thin sections and the lysigenous aerenchyma in mature tissue of crown root were observed using a cryo scanning electron microscope (cryo-SEM) with freezing fracture method. The schizogenous intercellular spaces in the root tip exist obviously in the middle portion of cortical cell layers close to the root-root cap junction, but not in root cap, stele and outer cell layers of cortex. The air spaces were formed at the junction of four neighbouring cells of inner cortex in the transverse sections, and between longitudinal cell layer connected along the root axis. Although many of those spaces were filled with liquid, some spaces seem to exist as air spaces. The lysigenous aerenchyma in the cortex, which hardly filled with liquid, emerged at 3~4 cm segment from the root tip and increased toward the basal region of root axis. The developing process of lysigenous aerenchyma was primarily separation of a radial row of cells caused by the shrinking and collapsing of cortical cells and then formation of septa along the radial cell rows by the fusion of cell wall with each other. These results suggest that the schizogenous and lysigenous aerenchyma play a role as a passage for the movement of oxygen into the root tip region where oxygen is required for respiration.

Key words : anatomy, aerenchyma, air space, cortex, rice, root.

It is well known that rice plants, likewise most species of wetland vascular plants, have well-developed aerenchyma in their body as an air transport system (Armstrong, 1971; Armstrong et al., 1991; John et al., 1974; Justin & Armstrong, 1987; Kawase, 1981). Well-developed aerenchyma in shoot and root can transport the air by diffusion from shoots to roots and rhizosphere where the molecular oxygen is utilized for aerobic respiration or oxidization of toxic substances, and thus allows rice plants to adapt to oxygen deficit conditions of a flooded soil (Armstrong, 1971; John et al., 1974). Those rice cultivars that have lower rates of oxygen flux via aerenchyma are susceptible to physiological injury associated with reduced soil conditions (Kawase, 1981; Lee, 1980). In some upland crops or non-wetland plants, aerenchyma formation in root is also induced under waterlogged or hypoxia condition as well as under ethylene treatment to roots (Kawase, 1981; Jackson, 1985). In most upland crops, there are highly positive relationships between the degree of aerenchyma development and flooding tolerance (Yamazaki, 1952).

Aerenchyma in roots usually forms either (i) lysigenously, by concomitant cell separation and collapse, or (ii) schizogenously, by separation of cell walls from each other without collapse (Armstrong et al., 1991; Esau, 1965). The development of root aerenchyma, especially in lysigenous aerenchyma, seems to serve following purposes; (i) it can greatly reduce diffusive resistance to longitudinal transport of gases from shoot to the roots, (ii) it results in a reduction in oxygen demand of root tissue per unit volume, and (iii) it may function to offset to some extent by lateral root development sub-apically (Armstrong, 1991; Kawase, 1981; Kawata, 1956; Kono & Yamada, 1972). Therefore understanding of aerenchyma development in crop plants may be useful in breeding for waterlogging or hypoxia tolerant plants.

In rice roots, well-developed lysigenous aerenchyma is observed in the cortex of mature root tissues, even when cultured aerated or without ethylene treatment, suggesting that its induction is genetically controlled (Jackson et al., 1985). Although anatomical observations on aerenchyma formation in rice roots have already been done by some researchers (Kawata, 1956; Kono & Yamada, 1972), further studies are needed to clarify, not only where the pathway of oxygen supply from developed aerenchyma in mature root region into a root tip exists, but also how the lysigenous aerenchyma in the region of mature tissue of crown roots develop.

In this study, we used the semi ultra-thin method and freezing fracture method with a light microscope and cryo-SEM to observe clearly the formation of the schizogenous and lysigenous aerenchyma along the crown root axis of rice plants.

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MATERIALS AND METHODS

Plant culture

Pregerminated seeds of a paddy rice (*Oryza sativa* L.) cultivar 'Milyang 23' were sown at a spacing of 3×4 cm on holes of covered iron mesh floating on water in plastic containers (L×W×H; 40×33×30 cm). The container was filled with half concentration of the Kimura B nutrient solution ($(NH_4)_2SO_4$; 48.2, KH_2PO_4; 24.8, KNO_3; 18.5, $Ca(NO_3)_2$; 59.9, $MgSO_4$; 65.9, Fe-EDTA $2H_2O$; 8.45 mg l^{-1}). The nutrient solution was renewed every week and adjusted to pH 6.0 using the HCl and NaOH solution, but not aerated. And the plants were grown under air temperature controlled conditions (day/night: 25/20℃) in a phytotron room under natural day length at Nagoya University from May to June 1993.

Light microscope observations

When the 8th leaves emerged from the 7th leaf sheath, the crown roots of the 3rd and the 4th phytomer (shoot unit) were taken from their stem, and 0.5 cm-segment of root tips were cut. For the semi ultra-thin sections, the excised root tips were fixed in a mixture of 3% glutaraldehyde and 1.5% paraform aldehyde in 0.1M cacodylate buffer (pH 7.2) for 4hr at room temperature, rinsed in 0.2 M cacodylate buffer and post fixed in aqueous 1% osmium tetroxide for 2hr. They were dehydrated through a graded alcohol series and embedded in Spur's resin. To make semi ultra-thin sections, the root segments were sectioned with glass knives on an ultra-microtome (Sorvall Porter Blum MT2-M) approximately 0.5 μm thick. After staining with toluidine blue, the sections were observed with light microscope and photographed.

Cryo-SEM observations with freezing fracture method

The axes of crown root from the 3rd and the 4th phytomer were harvested and cut into 1 cm segments from the tip to base. The length of harvested axes were in the range of 15~16 cm for 3rd phytomer and in the range of 10~11 cm for 4th phytomer. These segments were mounted on stubs and immediately frozen in liquid nitrogen, and then moved into a cryo-apparatus (Hitachi Cryo System) attached to a SEM (Hitachi S-2300). At low temperature by liquid nitrogen, the frozen segment were fractured with an iron knife attached inside of the cryo-apparatus. Soon after, the fractured face of frozen roots in the vacuum were observed using the SEM under low electron voltage at 1.5~2.0 kV. The SEM images were photographed on Kodak T-Max 100 films.

RESULTS AND DISCUSSION

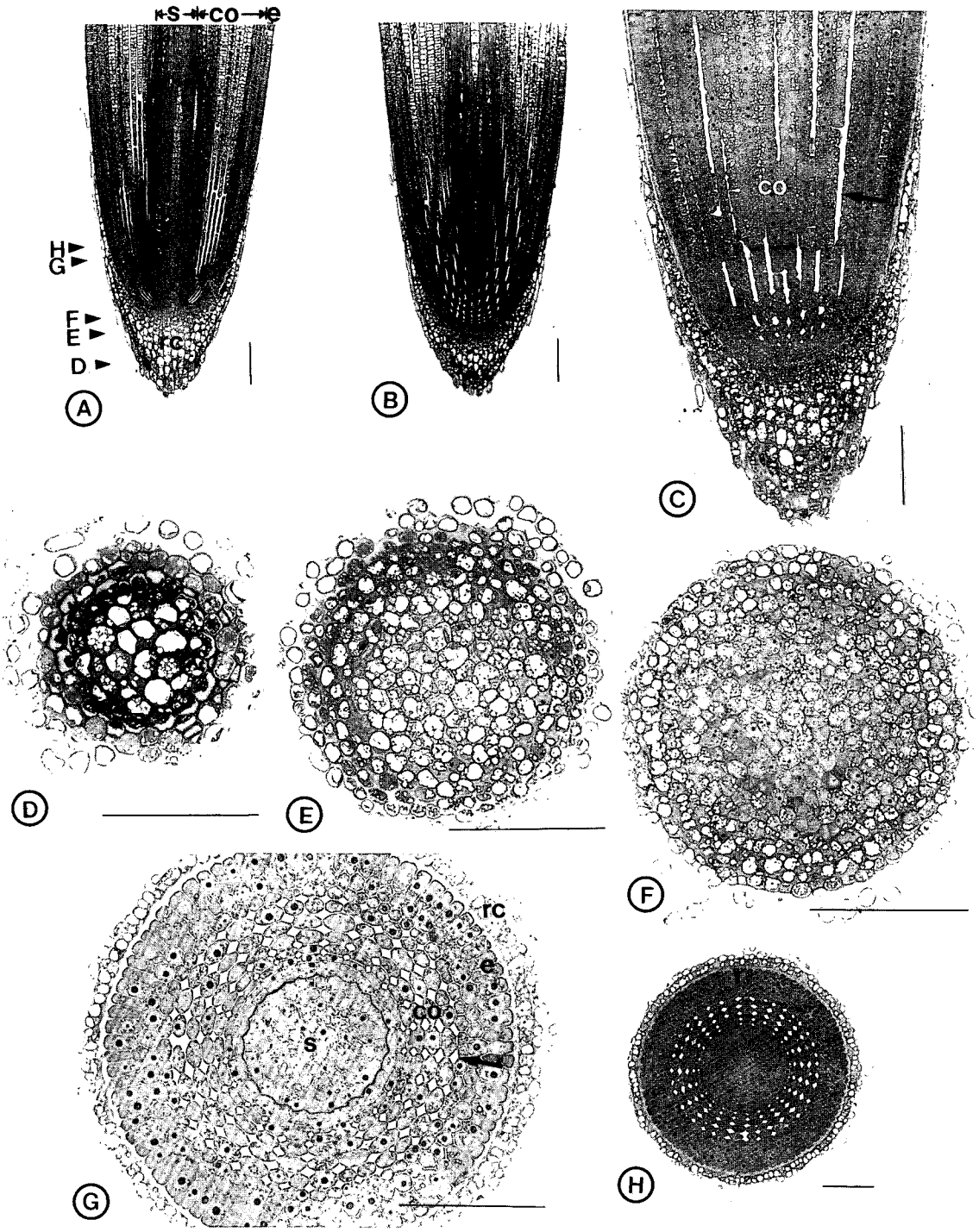
Development of schizogenous intercellular spaces (aerenchyma) in root tip

In longitudinal sections (Figs. 1-A~C), the intercellular spaces in the cortical tissue were usually formed between longitudinal cell layer and connected along the root axis. The intercellular spaces appeared close to the root-root cap junction where the cell layer of cortex seems to differentiate. Figs. 1-D~H show transverse sections of root cap (D~F) and root apical meristem region (G, H). The center portion of root cap showed columella cells containing starch grains which is thought to be a site of graviperception (Jackson & Barlow, 1981), and some cells were detached from outer surface of the root cap. In transverse sections of root tip, the schizogenous intercellular spaces were obviously shown in the middle portion cell layers of cortex, but not appeared in root cap and stele tissue (Figs. 1-A~H). These spaces formed at the junction of four neighbouring cells in inner cortex which columnarly arranged along the radial cell rows in the transverse sections. But the air spaces were not exist in outer cortex where the cells have the characteristics of hexagonal packing and differentiating stage.

Along a root axis of rice and other species plant the most active meristematic region is about 200~300 μm from the tip (Esau, 1965; Kawata & Matsui, 1977; Kawata et al., 1977). Water and salts absorption are relatively high between the root tip and branching site of lateral roots. And the radial oxygen losses and oxidation activity are also relatively higher on the surface of the root tip region than those in basal region (Armstrong, 1971; Luxmoore et al., 1970). Our observations by cryo-SEM (Figs. 2) and many previously reports (Armstrong, 1971; Jackson et al., 1985) indicated that cell collapsing and shrinking to form lysigenous aerenchyma in rice crown root was detectable in tissue of 2~4 cm region from the root tip and increased toward the basal portion.

From the above results, one question arises as to how air gas move from developing lysigenous aerenchyma into root apical meristem which need much oxygen for active metabolism. Our observation using the semi ultra-thin section showed that the schizogenous intercellular spaces formed at meristematic region very close to the root-root cap junction and longitudinally connected into the mature region (Figs. 1-A~C, F, G). From our observation by the cryo-SEM, the intercellular spaces seem to be filled with liquid or air gas (Figs. 2). Canny & Huang (1993) found with cryo-SEM in maize, much intercellular spaces from root tip to 10 cm region in cortex were filled with liquid. These results suggest that the intercellular spaces of rice roots filled with air play a major role in supplying oxygen into the root apical meristem region. Some spaces filled with liquid probably play an other important role of apoplastic movement of water absorbed in the root tip region, while immature xylem vessels yet have no role for water movement, as proposed in maize by Canny & Huang (1993).

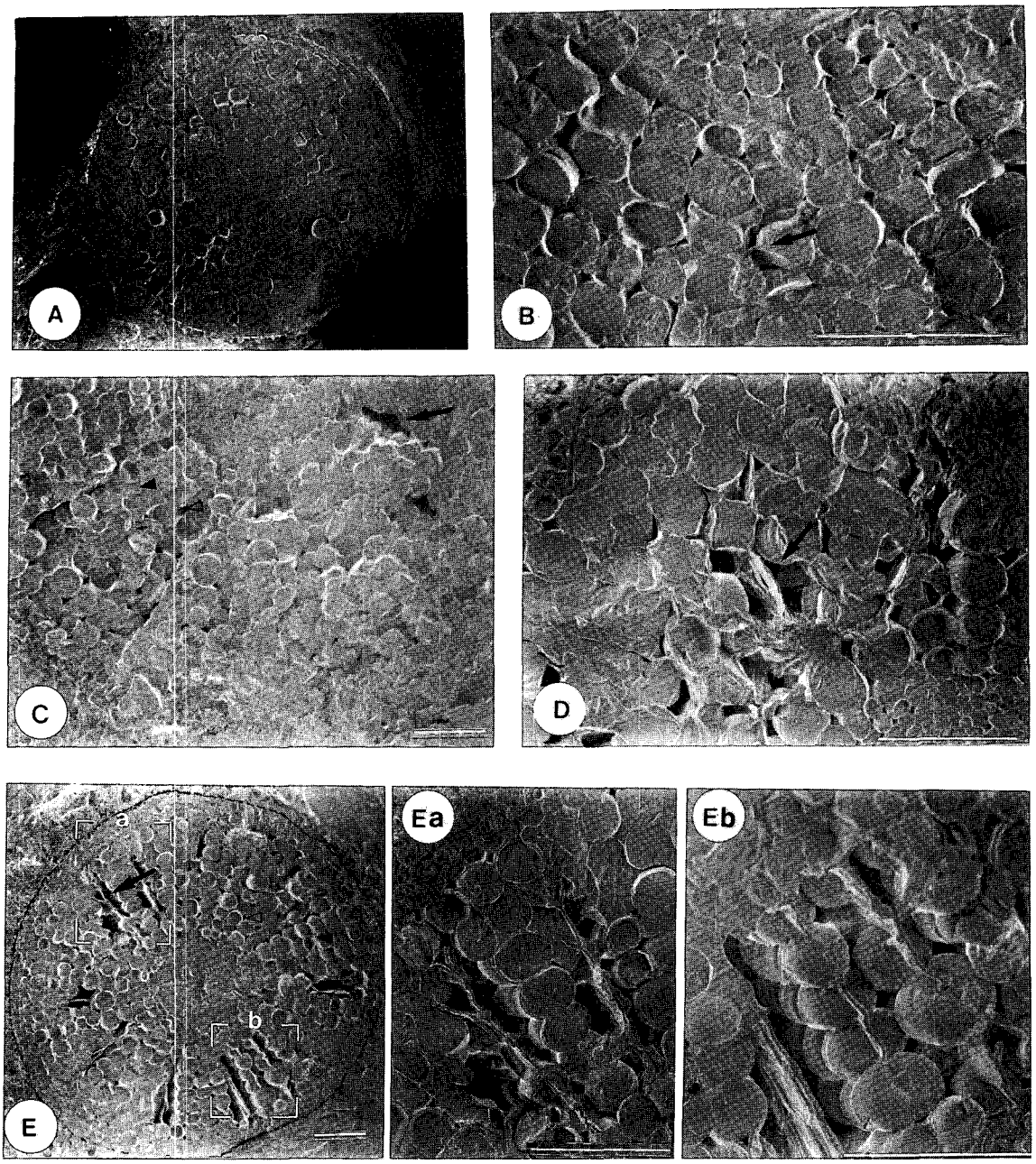
We reported that schizogenous aerenchyma was found in radicle of embryos of dry seeds, and increased in volume during germination using semi ultra-thin sections



Figs. 1-A~C. Various longitudinal semi ultra-thin sections of elongating rice root tip, at median portion (A), slightly oblique median portion (B) and outside portion of stele (C), showing the longitudinal connected intercellular spaces exist in cortex. In the Fig. 1-A, the arrow heads with alphabets D~H corresponded to the approximate levels of transverse section of Figs. 1-D~H, respectively. s: stele, co: cortex, e: epidermis, rc: root cap, black arrow: schizogenous intercellular space.

Figs. 1-D~H. Serial transverse semi ultra-thin sections of root cap (D~F) and apical meristem region (G, H) of rice crown roots, showing the radial arrangement of intercellular spaces which were formed at the junction of four neighbouring cells of inner cortex.

All vertical and horizontal bar sizes: 100 μm.



Figs. 2. SEM views of the serial transverse sections along the root axis from tip by the freezing fracture method showing the developmental process of lysigenous aerenchyma of crown root axes of the 4th phytomer at the 8th leaf stage in rice. The each distances of each fractional segment from tip of the crown root axis with mean length 10.5 cm: 1~2 cm(A), 2~3 cm(B), 3~4 cm(C), 4~5 cm(D), and 6~7 cm(E). White and black arrow heads show intercellular space filled with air or liquid, respectively. Black arrow shows the formation site of lysigenous aerenchyma. Figs. 2-Ea, b. Enlarged SEM view of each white rectangular zone in Fig. 2-E, showing the formation of septa along the radial cell rows by the fusion of cell wall. Bar sizes: 100 μm.

(Wada et al., 1996). All cortical cells of roots including exodermis, sclerenchyma and middle cell layers are differentiated by successive anticlinal and periclinal divisions from the dermatogen-periblem complex as a initial cells Kawata & Matsui, 1977; Morita & Nemoto 1995). These results indicate that the development of intercellular spaces arises by cell wall separation accompanied by cell division at the cortex of the root tip. Most of previously published reports (Kawata & Matsui, 1977; Kawata et al., 1977; Kono & Yamada, 1972), however, paid no at-

tention to the existence of intercellular spaces in apical meristem region of rice roots, because their observation were usually done using paraffin sections 10~15 μm thick where the intercellular spaces hardly emerged. In contrast, our observation of root tissue was done using semi ultra-thin sections of resin with 0.5μm thickness, and therefore we can clearly find the schizogenous aerenchyma in the apical meristem region of rice crown roots.

Development of lysigenous aerenchyma in the cortex of mature roots

Observation by a cryo-SEM of the segment with 1~2 cm from root tip, found schizogenous intercellular spaces in the cortex tissue as transversely fractured face of crown root, while the lysigenous aerenchyma was not shown (Fig. 2-A). Many intercellular spaces were filled with liquid and the size of those spaces seems relatively smaller than those observed by the semi ultra-thin and optical microscope.

The collapsing and shrinking cortical cell appeared on the 2~3 cm segment from the tip (Fig. 2-B). Developing aerenchyma appeared throughout the cortex tissue in the region of the 3~4 cm and 4~5 cm segments (Figs. 2-C, D). The development process of lysigenous aerenchyma seems as follows; initial separation from the neighbour cell rows caused by the shrinking and collapsing of cortical cells and then septa formed along the radial cell rows by the fusion of cell wall with each other. The formed lysigenous aerenchyma was shown as air space filled with gas, although some of schizogenous intercellular spaces seem to be filled with liquid (Figs. 2-D, F). Features of transverse sections from the 8~9 cm segment are shown in Fig. 3-A. About 40% of the radial cell row was collapsed and formed the radial septa. The fully developed aerenchyma is seen in the middle region of a crown root axis of the 3rd phytomer (Fig. 3-B) which is one lower phytomer than that in Fig. 3-A, at the same growth stage. Most of lysigenous aerenchyma space were filled with air, while some were filled with liquid.

In this study the development of lysigenous aerenchyma was also clearly observed by the cryo-SEM with freezing fracture method. The development of lysigenous aerenchyma in rice roots showed characteristics of formation of the septa in collapsed cortical tissue. The process was separation from the neighbouring cell rows caused by the shrinking and collapsing of cortical cells, and then septa formation along the radial cell rows by the fusion of cell wall with each other. These results are similar to those of Kono & Yamada (1972) and indicate that the lysigenous aerenchyma develop schizogenous as well as lysigenous process and then form the septum by the fusion of collapsed cell walls.

Because the development and function of aerenchyma in root of crop plants is quite important for adaptation to waterlogging and hypoxia condition, further ecological and molecular genetical studies with reference to development and function are needed to improve crop production in waterlogged or paddy fields.

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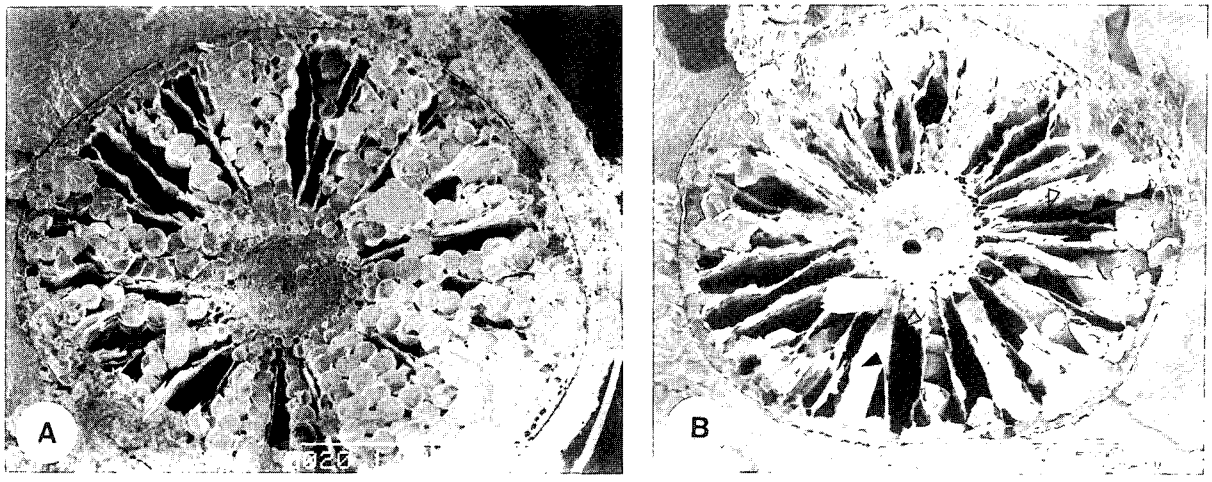

Figs. 3. SEM views on the transverse sections by the freezing fracture method showing the developing and developed lysigenous aerenchyma of crown root axes of 4th phytomer (A) and 3rd phytomer (B) at the 8th leaf stage of rice. The distances of each fractional segment from apex / mean total length of the crown root axes: 8~9 cm/10.5 cm (A), and 7~8 cm/15.5 cm (B). White and black arrow heads show lysigenous aerenchyma filled with air or liquid, respectively. Bar sizes: 500 μm.

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