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Potassium Distribution in the Apical Region of Rice Root

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ABSTRACT: Potassium (K) distribution in rice (*Oryza sativa* L.) root was studied by confocal laser microscopy, using potassium sensitive fluorescent dye potassium-binding benzofuran isophthalate (PBFI). Significantly high intensity of K-specific fluorescence was detected at the root cap region followed by meristematic and basal regions. A negligible or fainted fluorescence was observed at the root hairs area. These results suggest that K is heavily distributed in the apical area of rice root, which may be required in higher concentration for division and extension of cells, as it is the rapidly growing region of the root, moreover, may also be involved in water uptake by creating osmotic gradient across membranes.

Keywords: confocal microscopy, potassium-binding benzofuran isophthalate, PBFI, potassium, rice, root

Potassium (K) is an essential mineral nutrient and next to nitrogen, required in large amount for plant growth. If K is deficient or not supplied in adequate amounts, growth is stunted and yields are reduced. Potassium has been associated with essential role in plant water relationship apart from its specific role in enzyme activation, protein synthesis, photosynthesis, enhancement of rooting/early establishment and cell extension (Marschner, 1995). It is widely known for its rapid action as an osmotic regulator (Fischer, 1971). For example, it has been well reported that the movement of water in and out of guard cells is an osmotic response governed by K levels in the guard cells (Marschner, 1995; Moore *et al.*, 1995).

Rice is an important food crop in the world, especially in Asia. Therefore, numerous agronomic studies have been done for understanding and controlling development and growth of rice plants. However, information on rice roots is relatively limited. Although, rice root has different features from others, partly because it is usually grown under flooded condition, the structure of the root system is fundamentally the same as other cereals (Morita & Nemoto, 1995). In general, roots are integral components of nearly all plants. Root apex is the most active region in root. The primary apical meristem of the root produces the cells and tissue systems of the primary plant body, such as the epidermis, cortex, and primary vascular tissues (Russel, 1977).

The present work was based on the hypothesis that apical area of root is the most actively growing region; therefore, K must be present in a large quantity due to its requirement for the essential roles as an osmoticum and cofactor for enzymes. The distribution of K in rice root was investigated by application of potassium-binding benzofuran isophthalate (PBFI), using confocal microscopy which has been rarely applied to investigate the K distribution in plant cells (Rehman *et al.*, 2005). Therefore, one of the purposes of the current work was to investigate the application of PBFI and confocal microscopy for K distribution in rice root.

MATERIALS AND METHODS

Rice seedlings were grown in the experimental field of Chonbuk National University, Jeonju, Republic of Korea. Roots of six weeks old seedlings were used for K observation. Potassium-binding benzofuran isophthalate, a K^+-sensing fluorescent probe (P1267), was purchased from Molecular Probes (Eugene, OR, USA). The presence of K in the rice root was examined by loading PBFI as described by Halperin & Lynch (2003) and Rehman *et al.* (2005). The root of seedlings was placed in a 100 ml flask. The PBFI was dissolved in dimethyl sulfoxide (DMSO). Four millilitres of 20 μM of PBFI was added in the flask containing root. The flask was incubated in dark at 4°C for 1 h followed by incubation at 20°C for 1 h in dye free solution. The samples were observed under a confocal laser scanning microscope (Carl Zeiss LSM 510, Jena, Germany) with an optical filter BP 385-470 (excitation at 364 nm).

The intensity of K specific fluorescence was measured in pixels by using GAIA Blue image analyzer (http://www.gaia-zone.com). Each data point represents the accumulated value of randomly chosen ten points and their corresponding standard deviation values.

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RESULTS AND DISCUSSION

Fluorescent images of rice root resulting from the dye distribution of PBFI, illuminated with 364 nm light are shown in Fig. 1. The heavy intensity of fluorescence indicates the heavy presence of K, vice versa in rice root. It was found that the intensity of K specific fluorescence decreased with the distance increased from root cap to the basal region of root (Fig. 1E - H). Higher intensity of K specific fluorescence was observed at root cap compared to meristematic, basal and root hair regions. The root regions follow the order from higher to fainted fluorescence intensity as root cap > meristematic > basal > root hair region. Fig. 1I further elaborates the results, showing significantly higher concentration of K in root cap area compared to meristematic part which has significantly higher K than basal area of root. While a negligibly fainted K fluorescence was observed at the root hairs site (Fig. 1I).

It is known that in all kinds of roots, despite varying forms due to genetic or environmental factors, elongation depends on the division and subsequent extension of cells in the apical meristem. In majority of monocotyledonous plant species, including cereals and grasses, the entire root system develops from the apical meristem (Russel, 1977). The consistent appearance of K sensitive fluorescence has shown that K was heavily accumulated at the apical area of root. Similar results were reported by Pineda-Vargas *et al.* (2001). They reported that K was distributed heavily in the apical region of a forage crop *Brachiaria brizantha* root. Potassium is an essential macronutrient with a number of specific, irreplaceable roles in plants (Flowers & Lauchli, 1983; Leigh & Wyn Jones, 1984). Potassium plays a vital role as an osmoticum but is also involved in more specific metabolic roles including protein and starch synthesis and enzyme activation. Flowers & Lauchli (1983) concluded that Na can substitute for K in some functions, but not in all, and this substitution depends on the Na concentration. For example, Na accumulates in the cell vacuole and may substitute to some extent for vacuolar K. It cannot, however, substitute for K in its cytoplasmic functions (Leigh & Storey, 1991). Similarly, a large number of enzymes are either completely dependent on or stimulated by K. Potassium neutralizes the soluble and insoluble macromolecular anions and stablizes the pH between 7 and 8 which is optimum for most enzyme reactions. Potassium is required for protein synthesis even in higher concentrations than for

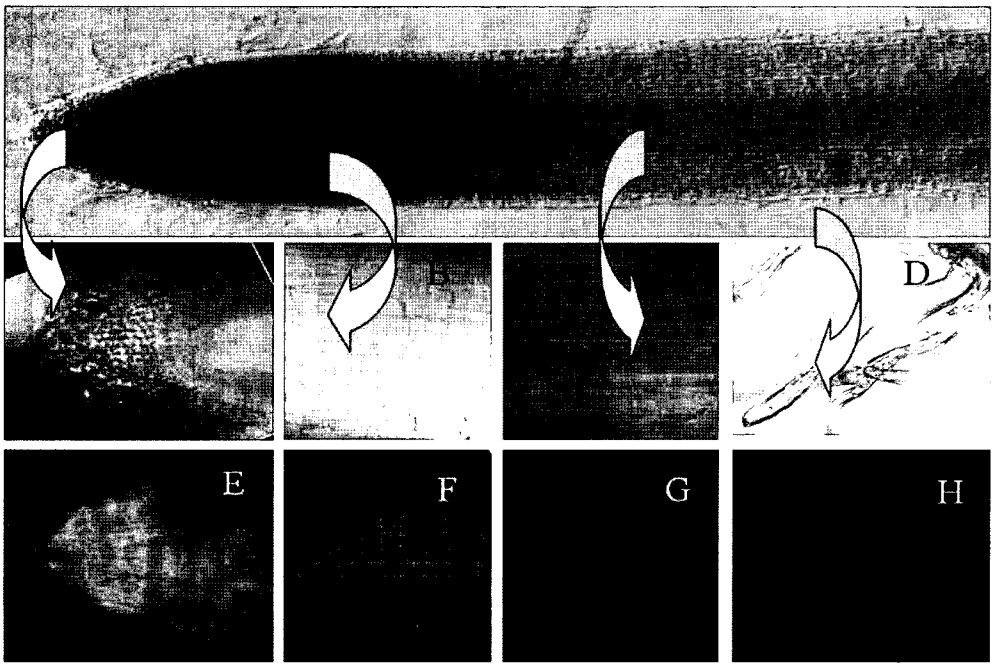

Fig. 1. Fluorescent image showing the distribution pattern of K in the rice root loaded with PBFI and excited with 364 nm light. The top most is the image of rice root and arrows indicate the sections of the root studied. A, B, C and D are bright field images of root cap, meristematic, basal and root hair regions respectively, while E, F, G and H are their corresponding fluorescent images. Root cap area (E) shows the heavy presence of K specific fluorescence. Meristematic region (F) shows comparatively less fluorescence than root cap but more than basal (mature) part (G) of root. While focusing at root hair (H) region shows a negligible fluorescence. Higher intensity of K specific fluorescence indicates the higher presence of K. The root was observed with a 40x water immersion lens (C-Apochromat, NA=1.2, Carl Zeiss) and an image was captured by a confocal microscope equipped with a 10x ocular lens.

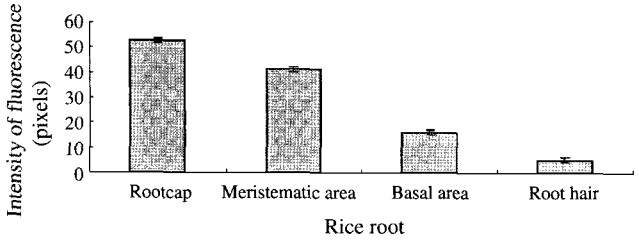

Fig. 2. The intensity of fluorescence measured in quantitative form (pixels) by using GAIA blue image analyzer in the rice root loaded with PBFI and excited with 364 nm light. Higher fluorescence indicates the higher presence of K which corresponds with the results shown in Fig. 1E-H.

enzyme activation (Marschner, 1995). For this reason, a deficiency of K in plants can lead to decreased metabolic functions and ultimately reduced plant growth.

Moreover, K is the most abundant cation in the cytoplasm and K and its accompanying anions make a major contribution to the osmotic potentials of cells and tissues of glycophytic plant species. For example, it has been well reported that the movement of water in and out of guard cells is an osmotic response governed by K levels in the guard cells (Marschner, 1995; Moore *et al.*, 1995). Therefore, the present results lead to the assumption that K could be one of the important factors regulating the water via the osmotic gradient created across membranes.

In conclusion, the current results shows that K was accumulated heavily in the apical region (root cap+meristematic) of rice root which may be required in higher concentration for (i) the division and elongation of cells due to its specific role in protein synthesis and enzyme activation and (ii) water absorption from the soil into the root system. Moreover, potassium binding fluorescent dye PBFI has been mainly used to measure intracellular K in animal (Kasner & Ganz,1992) cells and rare attempts have been made to use it for plant cells (Rehman & Yun, 2006). The present results proved that PBFI could become a valuable tool to determine K in plant root systems.

ACKNOWLEDGEMENTS

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